IMPERIAL MYCOLOGICAL INSTITUTE
34 AUG. 1936

THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY

Vol. 21, N.S., Nos. 27–34. JULY, 1936.

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[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,

BALLSBRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.,

20 NASSAU STREET, DUBLIN.

WILLIAMS AND NORGATE,

28 to 30 LITTLE RUSSELL STREET, STRAND, LONDON, W.C. 2.

1936.

Price Five Shillings.

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No. 27.

ON A RECENT BOG-FLOW IN THE COUNTY CLARE.

By G. F. MITCHELL, B.A.

[Read November 26. Published separately November 28, 1935.]

On Monday, October 29th, 1934, the Dublin papers reported the bursting of a bog in Killanena parish, Co. Clare, during the early hours of the previous morning. On the following Saturday (November 3rd), Professor L. B. Smyth and I arrived at the site and devoted that day and the following morning to investigation, of necessity somewhat cursory. The flow was not on such a large scale as those previously reported (1, 2), but some of the phenomena appear to be worthy of record.

The Slieve Aughty mountains, on which the bog rested, are composed of Devonian sandstone, with the underlying Silurian rock exposed through it in places, raised up above the surrounding limestone plain. At the south-west extremity of the range they reach their highest level (1,313 feet), and the disturbance extended to within 600 yards of this point. The strata forming the north-west slope of the hills dip gently in that direction at an angle of 3°, but the even rise of the incline is interrupted at intervals by escarpments, of which there are three above the 700-foot contour line. Below this level the course of the flow led it on to the underlying Silurian rocks. Save on the faces of these escarpments the flanks of the mountain above that level are covered uniformly with a blanket of peat. The surface flora included most commonly *Scirpus caespitosus, Narthecium ossifragum,* and *Eriophorum*: heather was not at all common except in the immediate vicinity of the drains, and *Sphagnum* though widely distributed was nowhere in large clumps. On the drier areas *Myrica Gale* was plentiful. The surface under-foot was fairly firm.

The burst had taken place at the edge of the highest escarpment (about 1,020 feet), and it is worth noting that a deep trench cut along the bog (marking a townland boundary) crossed the escarpment immediately to one side of this point. The width of the burst was estimated at 50 yards, and out of an area of at least this breadth and extending up the hillside for a distance of about 600 yards the bulk of the bog material had poured over the edge of the escarpment, leaving in many places the bare rock exposed beneath. Above this area disturbance had been less violent and only the more humified lower layers of peat had flowed out, the more solid crust subsiding and slumping forward somewhat, producing the long concentric tears described elsewhere

(1, p. 482; 2, p. 186), but not being carried very far forward. This flowing-out continued up to the highest point possible, *i.e.*, till stopped by the watershed at the summit.

The upper layers of the peat were fairly firm, being bound together by the roots of the existing vegetation, but the lower layers might be classified as 10 on the von Post scale (3) or completely humified, that is to say no plant structure was preserved, and on a piece of peat being squeezed between the fingers all the material passed out between them leaving nothing in the hand. The upper solid part or crust appeared to be about 3 feet in thickness, and, while it was difficult to estimate on the subsided margin the amount of softer material that had flowed out, it must have originally been at least 2 feet deep in most places. The lowest layer of all was more or less adherent to the underlying rock, and produced a greasy surface which was most treacherous to walk upon, but most suitable for the flow to pass over.

At the escarpment the material flowed over the edge, dropping about twenty feet, and then found itself on the gentle slope of the next shelf. This checked its velocity, and all the large sods or rafts of solid crust were dumped in a semi-circular area on the surface of the bog, which did not seem to have been cut into at any place. The piling up of these rafts immediately in front of the line of flow formed a dam round the sides of which the more liquid portions had to diverge. The flow of semi-fluid peat was thus divided into two branches which only re-united 700 yards lower down, though one branch covered in its circuitous flow about twice that distance.

A little way further down the hill two streams unite to form a tributary which flows into the Drumandoora river about two-thirds of a mile above the village of that name. As each of the stream valleys was encountered by one of the flows of peat, the flow became single again and continued on its way inside the river channel.

This it was enabled to do, as all the solid material had been checked immediately the debris poured over the escarpment, and only the more fluid parts proceeded further along the course of the river, inside the channel for the most part, but overflowing on either side whenever the river crossed a flatter area. It was, of course, in these level areas where spreadings occurred that soil had accumulated originally, and so the areas of spreading coincided exactly with such areas of arable land as were encountered by the flow. The margin of these extensions of the flow was always abrupt, being sharply convex, as was noticed in previous flows (1, p. 483). Not a great many acres were covered, but compared with the size of the holdings the losses were heavy, the fields being covered by about eighteen to twenty-four inches of peat of a porridgy consistency, through which potato-ricks and hay-cocks protruded here and there. The last spread of any account occurred where the tributary entered the Drumandoora at right angles, and though the level of the river rose considerably below that point, only the lightest flotsam was deposited by the flood on the low-lying riverside pastures.

While the immediate cause of the burst cannot be definitely ascertained, the terrain suggested strongly that a burst in this region was sooner or later inevitable. As the bog increased in thickness through growth, the depth of the humified lower layers also increased and tended to flow down hill under the influence of gravity: this, however, was prevented by the bank of well-drained firm peat on the top of the escarpment, which acted as a dam and held back the bog. But as the bog thickened the pressure on the bank unsupported on the other side increased also: it was bound sooner or later to reach a limit at which the bank would be pushed over the escarpment, thus releasing the lower layers.

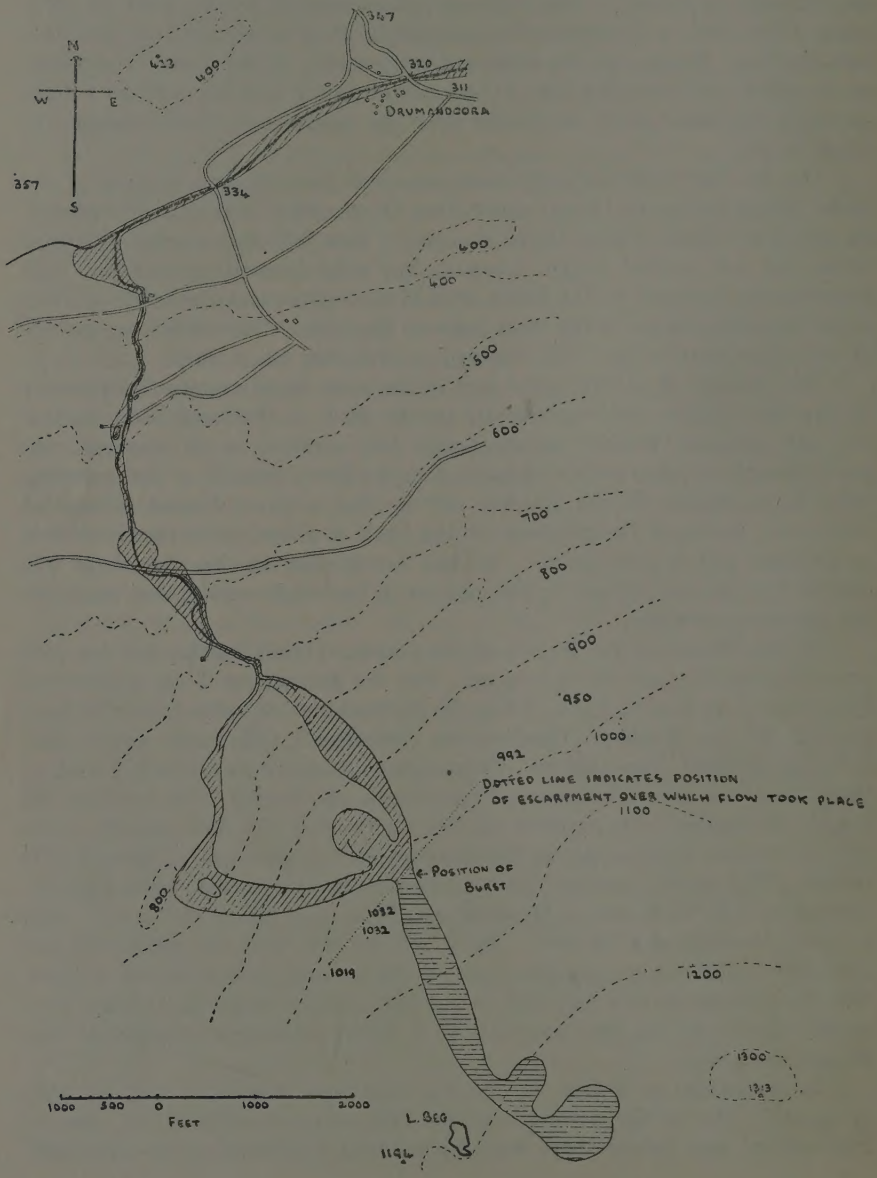
This is what happened: the bank gave way, and the first rushing of the under layers carried the firmer crust along in the form of rafts to be dumped on the flatter slopes below; but as the rate of flow fell off, the crust at higher levels was not carried bodily forward, but only lowered somewhat. This lowering was indicated by the fall in level of the surface and also by the welling up of the softer layers in the tears between the sods. This convex appearance of the softer material was also observed in 1931 flow (2, p. 186).

Any increase in weight on the part of the crust would increase the pressure on the lower layers and consequently on the bank at the escarpment, and so we may suppose, without entering into the question as to whether bogs are permeable or impermeable to water, that the heavy rainfall of the preceding week being unable to run quickly off the bog surface, formed of matted vegetation, increased the pressure on the bank to above the strain limit and so yielding had to take place. It does not matter whether the water was absorbed by the bog or merely retained on it, its weight would have produced the necessary pressure.

In this connection the records of the nearest rainfall stations for the week preceding the fall may be of interest. For the information I am indebted to Brig.-Gen. C. J. Hickie, C.M.G., Slevoyre, Borrisokane (22 miles from the flow), Lt.-Col. W. A. Bentley, Hurdlestown, Broadford (12 miles away), and J. C. MacLysaght, Esq., Raheen, Tuamgraney (10 miles away), and I wish to thank them for their assistance. From 9 a.m. on Sunday, 21st October, till 9 a.m. on Sunday, 28th October (a few hours after the flow started), there fell at Slevoyre 2·39 inches, at Hurdlestown 2·07 inches, and at Raheen 3·29 inches. The annual average fall at Slevoyre is 31·9 inches, with a weekly average of 0·62 inch, and at Hurdlestown the annual fall is 39·7 inches, with a weekly average of 0·76 inch. So it will be seen that the rainfall in this particular week was greatly in excess of the average, and it is not unlikely that the amount of rain that fell on the bog in these seven days stands in a causal relation to the flow, as a fall of 2 inches represents a weight of over 65 tons per acre.

In occurring at a face in the bog this flow resembled that of 1896 (1, p. 487), but in the present case the face was not artificial, *i.e.*, due to turf-cutting, and indeed there were no cuttings anywhere on the mountain-

side. The occurrence of a small tarn (see map) in the vicinity of the flow produced a superficial resemblance to the topography of the site of the 1931 flow (2, p. 183), and attention was directed to the lake to see if it was connected with or had been affected by the flow in any way. This was not the case, as



the disturbed area passed to one side and did not approach within 150 yards of it, nor did its level appear to have undergone any recent alteration. The lake was resting in a hollow in the peat surface.

In 1934 only one pine trunk was observed in the debris of the flow, and this relative absence of wood was commented upon. During a second visit in August, 1935, it was discovered that the upper layers of the flow had dried out and formed a skin on the surface on which it was possible to walk, though caution was necessary to avoid going through. It was then apparent that there was a certain amount of small wood fragments of Birch and Pine scattered through it, but nowhere in any quantity. This lack of the remains is in marked contrast to the two previously recorded flows, and must show that the sub-boreal forest cover on the Slieve Aughty mountains was an exceedingly thin one at an altitude of over 1,000 feet.

The flow was a quiet one which lasted irregularly over several days, a considerable volume of sludge descending, according to local witnesses, as late as Thursday, November 1st, four days after the initial burst. The only noise was due to the increase in volume of the water in the river, and this spate only differed from others in that the water was laden with decomposed peat which was deposited wherever the force of the current was checked.

My best thanks are due to Professor L. B. Smyth for his assistance in the field and helpful criticisms during the writing of the paper. I also wish to thank Dr. R. Ll. Praeger and Mr. A. Farrington who read the paper in proof form and made some suggestions, as well as the three gentlemen who loaned me the records of their rainfall stations.

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No. 28.

THE APPLICATION OF THE CATALASE TEST TO BUTTER.

By GEORGE CRUESS-CALLAGHAN, M.Sc.

[Read November 26. Published separately November 28, 1935.]

Catalase is an enzyme which splits hydrogen peroxide into water and molecular oxygen. Its activity in different samples can be compared by adding a standard quantity of hydrogen peroxide to a definite volume of the sample in a tube where the oxygen liberated can be collected and measured. Orla Jensen (1) states: "Almost all bacteria, with the exception of the true lactic acid bacteria, contain catalase, . . ."; elsewhere (2) he says ". . . and most of the putrefactive bacteria are particularly active in this respect, while the true lactic acid and the butyric acid bacteria do not produce catalase." Since excessive catalytic activity is sometimes due to a large number of bacteria or body cells, or to some other objectionable condition, the determination of the catalytic activity has been suggested as a test for the quality of milk and butter. The value of this test has been the subject of much controversy.

Quite recently Knudsen (3) published work relating to the application of the catalase test to butter. At first sight his figures would indicate that this test was of enormous value for determining the quality of butter, but as his figures are in conflict with the results of other workers it appeared of interest to investigate the matter further. As figures from work, probably on butter made from ripened cream, previously published by Knudsen are to hand, from which correlation coefficients between the catalase figures and the quality of butter can be worked out, it did not appear necessary to undertake work other than the statistical analyses of these figures already available.

When considering the use of the catalase test for butter two distinct points are at issue. These points are, firstly, the value of the test for determining the quality of butter, and, secondly, the use of the test for determining the keeping property of butter. When butter which has not been stored comes on the market it may, perhaps, be regarded as two weeks old, and to investigate the use of the catalase test for determining the quality of butter the points then allocated to the butter may be correlated with the catalase figure of the butter at this age. The use of the test for determining the keeping property of butter must be investigated by correlating the catalase figure with the loss or gain of points by the butter on keeping.

In 1930 Knudsen (4) published the results of work investigating the application of the catalase test to butter. In the first part of this work he shows the points awarded to samples of butters, and the catalase figures for the same samples two, four, and six weeks old. In the second part of the work he gives the catalase figures and the points awarded to 106 samples two weeks old. In the present communication these figures are analysed statistically,

both groups being used to determine the coefficient of correlation between the catalase figures and the quality of the butter. Of the 205 samples in the first group Nos. 183 to 205 have been excluded, as they appear to have been chosen on a different principle to Nos. 1 to 182. The full figures for 14 of these 182 samples are not available, so that in this group $n = 168$. For these figures $r_{cp}{}^{*} = - 0{\cdot}33$. From the other group which includes 106 samples $r_{cp}{}^{*} = - 0{\cdot}46$.

The figures in the first group have been used to ascertain the utility of the test for determining the keeping property of butter. The catalase figures of butters two weeks old have been correlated with the decrease or increase of points awarded to the butters after being kept from the age of two to six weeks. Here 162 samples are available, and give $r_{ck}{}^{*} = - 0{\cdot}44$.

Demeter and Christiansen (5) also working with butter made from ripened cream (6) tested "the catalytic constitution of butter artificially infected with harmful micro-organisms and stored for fifteen, thirty, and sixty days." When calculating a coefficient of correlation the method of sampling is of importance. It must be remembered that no variation in the method of sampling or in the treatment of samples within a group is permissible. Demeter and Christiansen appear to have used at least three different types of infection separately, and to have considered these along with samples not infected as one group. They give the correlation coefficient for samples thus treated for butters 15, 30, and 60 days old respectively as $- 0{\cdot}160$, $+ 0{\cdot}067$, $+ 0{\cdot}191$. Their conclusion, based on these low coefficients, "that the practical value of the test for the determination of butter quality is of little value," is therefore based on an erroneous foundation.

Osols (7) comes to the conclusion that the catalase figure must be regarded as unsuited for use in the judging of the quality of butter. Stören and Nestaas (8) as a result of trials with different organisms in butter, and varying percentages of salt, consider that the catalase test is not a reliable index of the content of foreign organisms in butter.

Knudsen in his paper of 1935, which has already been mentioned, gives the results from almost sixteen thousand samples of butter. He grades the samples into groups according to quality and gives the average catalase figure for the butters in each group. He claims a correlation coefficient for the better quality butter of $- 0{\cdot}85$, and for the poorer quality of $- 0{\cdot}96$. This is misleading because he has calculated these coefficients of correlation from averages instead of from individual samples. It need scarcely be pointed out that a coefficient of correlation thus calculated is quite useless as an indication of the *degree* of relationship between the variables. His figures merely show that there *is* a relationship between the catalase figure and the quality of the butter, but from them no deduction can be made as to the value of this relationship. Where any real correlation, however small, exists between two variables, if averages of groups are used the coefficient of correlation will increase with the number in the groups. In the limit, where averages of an indefinite number of samples

* c = catalase figure; p = points awarded to the sample; k = keeping property.

are used the correlation coefficient approaches ± 1. A coefficient of correlation properly calculated, that is worked out on the results of individual samples, not only indicates whether a relationship exists but also is a numerical measurement of the degree of this relationship.

In 1933 Knudsen and Jensen (9) put forward the view that defects causing butter to deteriorate are of two origins "bactériens" and "chimiques," the former being due to micro-organisms which produce catalase and the latter being due to micro-organisms which do not produce catalase and more frequently to purely chemical modifications. Accordingly, there are two factors which influence the quality of butter, and the general erroneous nature of the correlation coefficient – 0·96 is again evident from the fact that if it arises from one factor alone there is no residual effect possible for the other factor.

In conclusion, it appears that neither the very high coefficients of correlation between the catalase figure and the quality of the butter claimed by Knudsen, nor the very low coefficients put forward by Demeter and Christiansen are correct. A coefficient of about – 0·4, worked out from Knudsen's figures of 1930 is put forward as the most reliable index of the utility of the catalase test for determining the quality of butter. The same coefficient may be taken as the index of the value of the test for determining the keeping property of butter.

SUMMARY.

The value of the catalase test for the determination of, firstly, the quality of butter and, secondly, the keeping property of butter has been investigated. Reasons for the conflicting coefficients of correlation put forward by other workers, which vary from + 0·191 to – 0·96, have been given.

A coefficient of correlation between the catalase figure and the quality of butter of about – 0·4, and a coefficient of correlation between the catalase figure and the keeping property of butter of the same order, have been put forward as the most reliable index of the value of the catalase test when applied to butter. These coefficients have been worked out from figures, probably for ripened cream butter, previously published.

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No. 29.

THE MECHANICAL ASPECT OF THE NUTRITION OF FARM STOCK.

By E. J. SHEEHY, F.R.C.Sc.I., B.Sc.,
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[Read November 26. Published separately December 3, 1935.]

Prior to the second decade of the twentieth century research on animal nutrition was concerned with the determination of the nutritive value of the various foods available for stock feeding. Stimulated by the discovery of the dietary value of vitamins and "minerals," attention has since been focussed on the elaboration of a complete diet—that is, on the discovery of the ingredients necessary for rapid growth, full production, and satisfactory reproduction. The published records of the work done, dealing as they do with the relative utility of foods or with the response of the animal to feeding, discuss nutrition, for the greater part, from the point of view of chemical values.

Little attention has been paid by the scientific investigator to the effect of the physical constitution of foods on the response of the animal, though in practical feeding this is a primary concern. The opinion of feeders occasionally conflicts with that of the scientist, and frequently the results forthcoming from different research stations on the relative values of individual foods are at variance. It would appear as if these anomalies concern the mechanical aspect of nutrition, the bearing of which on the conduct of experiments designed to study the values of foods cannot be overlooked.

Quite a number of factors affect the physical character of a dietary, and the study of this aspect of nutrition necessitates the analysis of a complex of attributes. "Bulk" or bulkiness of a food refers to the space occupied by a definite weight of the material. This in turn is affected by a number of factors. Thus hay is described as bulky because of its large percentage of fibrous material and its consequent comparatively low nutritive value per unit weight. Conversely barley meal is concentrated. In comparison with cotton cake, coco-nut cake, because of its high absorptive powers, becomes very bulky when mixed with water. Though fibre is a major factor affecting "bulk," it is necessary to differentiate between the various types of fibre in this connection. Oats and bran have each about 10 per cent. of fibre, yet when moistened the bran becomes more bulky than the oats, and while steeped bran is laxative to the animal, steeped oats have not got this effect. Indeed, the same food prepared in different forms may exhibit different physical properties. Flaked maize and maize meal, though of practically similar chemical composition, differ in physical form, so that the flaked preparation is much more bulky than the meal.

Certain foods are bulky because of a very high water content, which imparts to them the property known as succulence. Uncooked roots and tubers and the moist fruits fall into this category. Succulent foods such as swedes have a laxative tendency, but yet when a quantity of dry matter, similar in composition and weight to that found in roots, is fed in addition to water the laxative effect of the roots is not reproduced. This laxative tendency, which in the animal body is associated with the promotion of good health and "bloom," is, however, a property not only of the succulent foods but also of many others. Cases in point are linseed cake and carrageen moss foods which appear to exercise a lubricative agency in the gut: liquid paraffin is a truer type of lubricant.

These diverse physical properties are connected with the utilisation by the body of the nutrients of foods. Rations must be so constituted that a correct mechanical condition obtains, and in the case of experimental work on the value of individual foods it is necessary to ensure that, in the determination of the comparable value of their chemical ingredients, a uniformity of physical characteristics in the comparable diets be maintained.

Experimental.

Experiments which have been in progress for some years in the Animal Nutrition Department of University College, Dublin, incidentally brought out certain features which bear on the mechanical aspect of nutrition. Specially designed experiments, chiefly on pigs, investigated other features, and the observations and results, together with a discussion of their bearing on food utilisation by the animal, and on the interpretation of the results of feeding experiments, are presented in this paper.

Linseed Cake. (Pigs.)

The popularity of linseed cake for cattle and sheep feeding is widespread. In certain districts linseed cake also forms part of the pig's diet, and users claim that its inclusion ensures health and thriftiness in the animals. To test the validity of this claim three experiments were conducted on pigs.

Experiment I.

In the first experiment two groups of animals were fed for a period of 16 weeks. Each group contained 7 pigs of an average initial weight of 42 pounds, and the following rations were fed:—

	Group A.	Group B.
Yellow maize meal	68	53
Barley meal (app. 6 per cent. fibre)	22	17
Linseed cake meal (app. 9½ per cent. fibre) ...	—	20
Meat meal (including bone)	12	12
Salt	1	1

The food was moistened with water previous to feeding. The animals were housed, but were given exercise regularly, and sunshine was admitted to the pens. No litter was provided, the pigs' sleeping quarters being floored with wood. Both groups were fed according to appetite.

Throughout the entire period of the experiment group B consumed appreciably more food than A. The weekly live-weight gains of the pigs in group B were consistently nearly 50 per cent. greater than those of A, and over the entire feeding period the number of pounds of food fed per each pound of gain was 3·66 and 2·97 for groups A and B respectively.

There was a very considerable difference in the volume of the fæces voided, those from A being small and dense, while B's were much larger and softer. This feature of the experiment was more noticeable while the pigs were young than it was in the later stages of the experiment. The individual pigs of group B grew more uniformly than those of A, and at all times looked healthier.

Obviously the replacement of part of the maize and barley by the linseed cake meal enhanced the value of the ration very considerably. An ample supply of protein was provided to both groups, so that the special value of the linseed cake meal is to be credited to its oil or fibre content or to lubricative properties associated with other constituents. The consistency of the fæces showed that the contents of the lower gut of group B animals were more fluid than those of A.

Experiment II.

Two groups (8 in each) of pigs of an average initial weight of 37 pounds were treated as in Experiment I and fed the following rations:—

	Group A.	Group B.
Yellow maize meal	50	45
Pollard (app. 6 per cent. fibre)	40	30
Linseed cake meal (app. 9½ per cent. fibre) ...	—	15
Meat meal (including bone)	12	12
Ground limestone	1	1
Salt	1	1

As in Experiment I, group B consumed more food than A. The weekly gains were in favour of group B for the first half of the period, but owing to greater progress of the larger pigs in group A towards the end, the average group gains of both were similar for the second half of the feeding period. The food cost per pound of gain was 3·21 and 3·10 for A and B respectively.

During the early weeks of the experiment the fæces from group A were denser, harder, and less bulky than those of B. This feature was particularly noticeable in the case of the smaller pigs of group A, which suffered a mild form of chronic constipation, and which evidently were at a greater disadvantage than the larger animals in the utilisation of their concentrated diet. As the pigs advanced in age, the difference in the character of the fæces between the two groups tended to disappear.

While, however, the members of group B grew and developed uniformly, those of group A showed differentiation in progress and in ultimate size and weight.

In this experiment the value of the ration was, by the inclusion of linseed cake meal, enhanced to a lesser extent than in Experiment I, so that ration A of Experiment II is superior to ration A of Experiment I. Presumably the greater quantity of fibre and the possible superiority of the fibre of the pollard over that of barley supply an explanation.

Experiment III.

Two groups (average initial individual weight—28 lb.) of 8 pigs, each treated as in Experiments I and II, were fed for 15 weeks on the following rations:—

	Group A.	Group B.
Yellow maize meal	25	25
Pollard (app. 6 per cent. fibre)	40	25
Bran	25	25
Linseed cake meal (app. 9½ per cent. fibre) ...	—	15
Meat meal (including bone)	12	12
Salt	1	1

Both groups ate similar quantities of food, and each made an average weekly gain of approximately 9 pounds. The progress of the two groups was similar throughout the experiment, and the consistency of the fæces was soft, there being no difference between the groups in this respect.

The presence of 25 per cent. of bran in the rations used in this experiment deprived the linseed cake meal of any beneficial effect, and this result, taken in conjunction with those from Experiments I and II, shows that the value of linseed cake meal in a pig's dietary depends on the constitution of the remainder of the ration. The standard ration in each experiment, i.e., ration A, was, as far as information is available, chemically complete, so that the effects of the experimental diets are to be considered in the light of their mechanical action in the alimentary tube of the animal.

Oil. (*Pigs.*)

Notwithstanding the fact that linseed oil is fairly completely assimilated, there remained the possibility of the effect of the linseed cake meal being attributable to its oil content. Two experiments to test this were conducted.

Experiments IV and V.

In each there were 2 groups of 6 pigs each, the average initial weight being 31 and 45 pounds in the first (Exp. IV) and the second (Exp. V) experiments respectively. The animals were housed, bedded with straw, and were given an opportunity for exercise and light irradiation. The control rations were so constituted as to contain a minimum of oil and consisted of

blood meal, extracted soya bean meal, pollard, barley meal, bran, ground limestone, and salt. In Experiment IV 1 per cent. linseed oil and in Experiment V 2 per cent. was included in the experimental ration.

No difference between the control and the experimental ration was indicated either in food consumption or economy of gain, and the health of the comparable groups was similar, so that it would appear as if the oil was not a major factor in the enhancement of a certain type of ration by linseed cake meal. An interesting observation was made, however, in the case of Experiment IV, where the diet was costive in character. While both groups consumed portion of the straw provided as litter, that which received the oil showed a lesser tendency in this respect than the other, suggesting that the oil helped to counteract the ill-effects of the costive diet, even though such was not apparent in the weight increment or in the economy of food utilisation.

Oats versus *Linseed Cake Meal.* (*Pigs.*)

Experiment VI.

Should the linseed cake meal influence the feeding value of a ration by virtue of its fibre *per se,* then any other food, oats for instance, containing a similar amount of fibre should exercise a like effect. The investigation of this point formed the motive of Experiment VI, in which the following rations were compared:—

	Group A.	Group B.
Yellow maize meal	58	58
Barley meal	12	12
Oats—ground (app. 9½ per cent. fibre) ...	20	—
Linseed cake meal (app. 9½ per cent. fibre) ...	—	20
Meat meal (including bone)	12	12
Salt	1	1

Each group contained 6 pigs, the average initial live-weight being 38 pounds. They were housed continuously on bare floors, and because of the dark winter weather which prevailed during the test codliver oil was fed at the rate of ½ ounce per pig daily. The experimental diet was thus made chemically complete, so that the oats and linseed cake could be judged from their mechanical effect. The experiment continued for 11 weeks.

From the beginning it became apparent that group B would consume more food than A, and, consequently, they were each fed according to appetite. Over the feeding period the pigs of group B ate 25 per cent. more food than A, they made considerably greater gain, and the food cost per pound of gain was 4·1 lb. and 3·0 lb. for A and B respectively. Ration A proved costive, especially for the smaller pigs, the fæces of which were comparatively hard and of small dimensions, and frequently evacuated in pellets rather than in the normal pudding form. The costive nature of the diet reacted so adversely on the progress of group A that after 7 weeks it was considered advisable to alter

the ration, so as to counteract the constipative effect. Accordingly, 10 per cent. sugar pulp (molassed dried beet pulp) was added to each ration for the remainder of the period. Notwithstanding this change group A still made less progress than B, and it appeared as if the smaller pigs, at any rate, had suffered a more or less permanent disability as the result of the earlier dietary.

It is clear from this experiment that the presence of a certain amount of fibre does not ensure a correct physical condition of the ration for a pig. Fibre alone may provide for optimum conditions in the stomach; but, in order that the lower gut may function properly, either the fibre must have certain favourable properties, or, alternatively, the food must contain other constituents which favour healthy activity in that organ.

Bran in the Ration. (*Pigs.*)

Unlike oats, bran absorbs water freely, and expands in doing so, and the substitution of bran for oats in the standard diet of Experiment IV would have changed the physical condition of the diet, and made its mechanical effect very different from what it was. This special property of bran is indicated in the following experiment. The question arose, however, as to how far the inclusion of bran in an otherwise concentrated ration would prove beneficial, and at what stage excess of bran would operate adversely. An experiment was arranged to investigate this matter.

Experiment VII.

Four groups of 8 pigs each, and of 55 pounds individual initial weight, were housed on bare floors without litter: exercise was provided by allowing access to yards in the sunlight, and the rations fed were as follows:—

	Group A.	Group B.	Group C.	Group D.
Yellow maize meal (app. 2 per cent. fibre) ...	60	50	40	30
Barley meal (app. 5½ per cent. fibre) ...	30	30	30	30
Bran (app. 9½ per cent. fibre)	—	10	20	30
Meat meal (including bone) (1 per cent. fibre)	12	12	12	12
Salt	1	1	1	1

Each group got exactly the same quantity of food, which was regulated by the appetite of the slowest feeders, and which was fed in the wet condition. The experiment continued over 12 weeks, and, as in all the previous tests, the individual animals were weighed weekly.

The average live-weight gains per week for the full feeding period were 8 lb., 8½ lb., 8½ lb., and 8 lb. for groups A, B, C, and D respectively. The weekly live-weight increase and the appearance and health of the animals throughout provide more information than do the average weight increments for the experimental period. The animals in group A became constipated, and it was found necessary to dose a number of them with glauber salts on

three or four occasions. The constipation was most pronounced in the early weeks of the experiment, and while it was still present about the middle of the period, it practically disappeared towards the later stages. None of the other groups showed any traces of digestive disorganisation. The fæces of group A were hard, and of small size, while those of group D were very bulky and soft. Groups B and C were intermediate in this respect. Throughout the experiment groups B, C, and D were particularly healthy, and grew somewhat quicker than group A; but, while groups B and C, particularly B, fattened more or less normally, group D remained thin, and at the termination of the test none of the pigs in that group was fit for slaughter. On the other hand, group A showed less growth but more fat development than any of the other groups. Though an opportunity did not offer itself for an examination of the carcases, it was clear that when the experiment closed the pigs of group A were fatter than those of B, but from outward appearance the latter promised to make more desirable bacon carcases.

The concentrated diet of group A proved a limiting factor to their progress: first, in so far as its costive effect, if not counteracted by the salts, would have caused fatalities. Secondly, it tended to produce a smaller and fatter pig than the trade desires. Thirdly, it definitely limited appetite, and if, instead of being fed a like quantity of food, the four groups were given as much as each would consume, groups B, C, and D would have eaten much more, and would obviously have reached very much greater weights. Judging from the results of earlier experiments, the economy of food consumption would also have been greater.

An interesting observation was that the constipation of group A was less pronounced when the food was fed in a very sloppy condition rather than in the consistency of dough.

The condition of the fæces showed that, unlike oats, bran absorbs and holds, even in the lower gut, a considerable quantity of water, thereby maintaining the tube dilated, and possibly more elastic, and thus helping the onward movement of the gut contents and promoting regular evacuation.

The results of Experiment VII show that 30 per cent. of bran is excessive in the diet of the pig, but that the addition of 10 per cent. of bran to an otherwise very costive and concentrated diet maintains health, and promotes "bloom" in the animal. Apparently the optimum proportion of bran in the mixture used in this experiment would have been about 12 per cent., or, better still, 20 per cent., in the early stages and 10 per cent. later. Such a mixture with 20 per cent. of bran would contain about $4\frac{1}{2}$ per cent. of fibre, and with 10 per cent. of bran $3\frac{3}{4}$ per cent. of fibre.

Lubrication. (*Pigs.*)

Experiments VIII and IX.

Attempts were made to rectify a costive concentrated diet by adding thereto a lubricant, the pigs being necessarily confined on a bare floor. In the first

case carrageen moss was used, and in the next trial liquid paraffin. The history of both experiments was similar. The animals on the normal diet suffered the disabilities referred to in the case of the very concentrated rations used in earlier experiments. They appeared very restive, and spent much of their time roaming round the pen, nosing the trough, rooting at their own fæces, and endeavouring to chew the partitions. The addition of the carrageen moss and of the liquid paraffin to the experimental groups caused much of this restlessness to disappear, and changed the character of the fæces by rendering them softer and more bulky. Apparently the effect, though less pronounced, was similar to that produced by bran and linseed cake meal, and was concerned with the condition of the gut contents, particularly that of the lower part of the colon and rectum. After some weeks, however, the effect of the addition, both in the case of the carrageen and liquid paraffin in the quantities used, wore off. The amounts fed were then increased in an effort to promote a continuation of the beneficial effect of the smaller initial supplement, but, unfortunately, a stage was reached when the experimental rations became distasteful, the appetite slacked, and all endeavours to induce the pigs to consume the larger quantities failed.

It would appear that a lubricant, such as liquid paraffin, temporarily at any rate, enhances the value of a mechanically incorrect ration by preventing sluggishness of the lower gut.

Oats in the Ration. (*Pigs.*)

Experiments VI and VII showed that oats to the extent of 20 per cent. does not correct a costive diet to the same extent as 10 per cent. of bran. Presumably, however, the addition of oats confers some benefit on an otherwise very concentrated diet. Otherwise, because of the addition of the rather indigestible fibre in oats, there should be a depression of the nutritive value of the diet. The following three experiments relate to the elucidation of this problem:—

Experiment X.

Two groups of 8 pigs each—80 lb. average initial weight—were placed on bare floors, given exercise and sunshine, and fed the following rations for 8 weeks:—

	Group A.	Group B.
Yellow maize meal	70	55
Barley meal	20	20
Oats—ground	—	15
Meat meal (containing bone)	10	10
Salt	1	1

Both were given the same weight of food, which was fed wet, daily.

The live-weight increase, which was low, proved to be the same in both groups, in both of which also the fæces were hard, and of small size. There was no actual constipation, as occurred on similar diets in earlier experiments, but an explanation of this lay in the greater initial weight and size of the experimental animals.

Experiment XI.

Oats having in the previous experiment proved equal to maize when added to a very concentrated ration, it was next tested in a ration which from the mechanical point of view was suitable. Two groups of 6 pigs each—average initial live-weight 42 pounds—treated as in Experiment X, were fed the following mixtures:—

	Group A.	Group B.
Yellow maize meal (1·8 per cent. fibre) ...	40	10
Barley (4·5 per cent. fibre)	10	10
Pollard (6·5 per cent. fibre)	20	20
Bran (8 per cent. fibre)	20	20
Oats—ground (9·2 per cent. fibre)	—	30
Meat meal (containing bone) (0·8 per cent. fibre)	10	10
Salt	1	1

Both groups were given the same daily quantity of food, which was fed in the wet condition. The experiment continued for 20 weeks.

Similar progress was made by both groups for the first 8 weeks, after which group A did consistently better than B, the average live-weight gains over the entire period being 10 and 9 pounds per week in groups A and B respectively. Each pound of gain cost 3·04 pounds of food in the case of group A and 3·36 pounds in B. There was little or no difference between the groups in respect of the fæces, which were normal, while both groups were particularly healthy throughout. At the termination the skin of the pigs in group B showed less "bloom" than those of A, the feel on the surface was rougher, and the hair was coarser and more in evidence. Group A animals were fatter than those of B.

It is clear from this experiment that, when oats is tested against maize in a ration which is already mechanically suitable, the maize is a superior food-stuff. It induces a greater live-weight gain, the gain is less costly of food, and the carcase of the animal carries more fat. This is in direct contrast with the result of Experiment X, in which the oats was added to a diet mechanically unsuitable because of excessive concentration.

Experiment XII.

In view of the results obtained from Experiments X and XI another test on 4 groups of pigs was conducted, the effect of the substitution of oats for

part of a moderately concentrated ration (A) and for part of a bulky one (C) being investigated. The following were the rations fed:—

	Group A.	Group B.	Group C.	Group D.
Yellow maize meal (1·8 per cent. fibre) ...	38	8	38	8
Barley meal (5 per cent. fibre)	12	12	12	12
Pollard (4·9 per cent. fibre)	40	40	20	20
Bran (9 per cent. fibre)	—	—	20	20
Oats—ground (9·2 per cent. fibre)	—	30	—	30
Meat meal—containing bone (0·8 per cent. fibre)	10	10	10	10
Salt	1	1	1	1

Eight pigs were included in each group, the average initial weight being 28 pounds. The experiment was continued for 23 weeks. Similar weights of food were fed to all groups.

The average weekly gains over the entire period for groups A, B, C, and D were 8·80, 8·85, 9·0, and 8·0 respectively, the food costs per pound of gain being 3·29, 3·28, 3·24, and 3·64. The fæces from groups A and B were, in consistency, more or less alike, and were less bulky and denser than those from C and D, which were similar to one another. The results from groups A and B showed that on a live-weight increment basis oats worked out similar to maize, but the animals in group A were from external appearance fatter than those in B. There is the possibility of group A having accumulated more energy in its body, but for purposes of bacon production the pigs in group B appeared superior. Though the results from group C were not significantly superior to those from A and B, yet the difference was in favour of C, which group looked better and possessed more "bloom" than either A or B. Possibly, in comparison with A and B, C would have done better were the proportion of bran in the ration less. It would also have consumed more food were each group fed to appetite: the optimum mechanical condition was possibly over-reached by the inclusion of bran to the extent of 20 per cent. Group C made definitely more progress, and was more economical of gain than D, so that the addition of oats to a diet, constituted as in group C, definitely reduced the nutritive value of the ration.

Green Feeding. (*Pigs.*)

Experiment XIII.

Five groups of 7 pigs, each of an average initial live-weight of 38 pounds, were all fed as follows:—

Group 1 got a concentrated costive diet, group 2 a somewhat similar diet, which, while concentrated, yet did not prove costive; group 3 the same diet as group 2, supplemented by a quantity of trough-fed green food; group 4 a ration which differed from that fed to 2, in that some of the concentrated ingredients

were replaced by 20 per cent. of bran; and group 5 a similar ration to 4, supplemented by codliver oil. The percentages of fibre in rations 2 and 4 were 3½ and 5 respectively.

The meals in the case of all groups were so chosen as to contain a minimum of vitamin A, which was provided to groups 2 and 5 in the forms of green food and codliver oil. All the groups had the advantage of abundance of sunshine: they were housed on bare floors.

The pigs of each group were fed according to appetite. Groups 3, 4, and 5 ate a similar quantity of meals; group 2 ate less in the latter part of the experiment, while group 1 ate less than the others from the beginning. Comparison of the rates of gain of groups 4 and 5 showed that towards the end of the experiment the want of vitamin A in the ration of group 4 prevented it from making the same progress as group 5. Group 1 became constipated, and, though treated, it made the least progress and showed the least economy in food utilisation. Group 2 made about the same progress as 4 during the first three-fourths of the experimental period. Thenceforward, when the food consumption of the pigs had reached a high level, group 2 ate less than 4, and the rate of growth was consequently slower. Some three pigs in group 2 suffered from digestive trouble three or four times in the later stages of the test. Evidently the ration of group 4 was somewhat superior to that of 2 and much superior to that of 1 from the mechanical point of view. Group 3, receiving the supplement of grass in addition to a fairly concentrated meal mixture, did equally well with group 5, suggesting that the grass served not alone the part of codliver oil in supplying vitamin A, but also that of bran, in correcting the physical unfitness of the meal mixture fed along with it.

Discussion.

In certain of the experiments on oat feeding, in which the energy value of the ration was considerably reduced by the addition of oats, no reduction in the nutritive value of the ration, as judged by live-weight increment of the animals, took place. Hence the reduction in energy value was compensated for by an enhancement of the nutritive value of the ration in some other fashion, and since there is no reason to believe that the standard ration was deficient in any chemical ingredient, it may be concluded that the addition of the oats improved the mechanical condition of the mixture fed. Possibly most of this beneficial influence operated in the stomach, though the presence of the oats may have had a favourable influence on the small and large intestine also. The results show, however, that when the ration is already properly constituted from the mechanical point of view, the addition of oats depresses its nutritive value. In that case the energy value of the ration is reduced without any counterbalancing beneficial influence. Experimental work conducted by the Department of Lands and Agriculture (Irish Free State) (1), Blissett (2), Carroll (3), the National Research Council, Dominion of Canada (4), and by

Wright (5) shows that oats may replace more concentrated meal in the ration of the pig to the extent of 25 to 33 per cent. without reducing the efficiency of the ration. In many of the tests conducted the initial weight of the animals reached 60 to 70 pounds, which corresponds with an age beyond which pigs utilise oats better than they do in early life. Observations made by the writer on groups of pigs receiving crushed or rolled oats showed that in young pigs the oat retained in the fæces the form in which it was fed, while as the animal grew, the oat gradually disappeared from the fæces, till finally it could no longer be discerned by naked-eye examination. The effects of the replacement of other foods by oats in the pig's diet depend on the age of the animal as well as on the constitution of the remainder of the diet.

It would appear from the experiments reported that one of the chief merits of linseed cake lies in its mechanical effect. Animals, whether it be the horse, sheep, or pig, whose diet contains a moderate proportion of linseed cake are free from digestive disorganisation, the fæces are evacuated regularly, and have what the feeder describes as a healthy consistency, the appetite of the animal is well maintained, and the skin and coat exhibit the property known as "bloom." While, of course, such conditions cannot obtain in the absence of a complete diet, it would appear as if the popularity of linseed and linseed cake is due to features which are concerned with the mechanical rather than the chemical aspect of the diet. Other foods, however, produce the same desirable mechanical effect in the alimentary tube, and where the ration already contains sufficient of such foods then linseed cake, in comparison with other cakes and meals, has no special merit for farm animals. Linseed cake contains some of the mucilaginous coat of the flax-seed, from which it is prepared, and unless the fibre of the linseed cake has special water-holding properties not possessed by that of oats, then the particular merit of linseed cake lies in the mechanical effect on the alimentary canal of the mucilaginous constituent.

The experiments with oats—e.g., X, XI, and XII—and also those with linseed cake—e.g., I, II, and III—show the necessity in experimental work for having regard to the mechanical condition of the ration, and the possibility, if this is neglected, of arriving at incorrect conclusions. The physical nature of the control ration determined in these experiments a superiority or equality of linseed cake meal to maize or pollard, and an equality or inferiority of oats to maize meal for pig-feeding. The conditions under which it is fed also determine the nutritive value of bran. Kellner (6) gives bran a starch value of 43 as compared with 72 for linseed cake, but Crichton and Allcroft (7) find that the value of bran in the ration of the ox is higher in relation to linseed cake than the respective starch equivalent figures indicate. Diverse opinions on the value of bran were expressed by an outstanding scientist and a remarkably successful practical feeder at a meeting of the British Farmers' Club (8). The special value of bran for the prevention of constipation in the human is shown by experiments conducted by Cowgill and Sullivan (9) and also by Cowgill and Anderson (10), who, in discussing the physiological roughage minimum for

the human, showed that the value of bran was greatest when the bran supplemented a mildly constipative diet. That the effectiveness of bran in this respect is largely due to the nature of its fibre is shown by Olmsted, Curtis, and Timm (11) in work also done on the human subject. The results obtained by the writer, both in the case of pigs and cattle, show that bran is an eminently valuable food for the establishment of optimum mechanical conditions in the alimentary tube. Not alone has it a very favourable effect on the activity of the lower gut, but on account of its flakiness it promotes healthy activity and rapid digestion in the stomach. It would seem as if its special merits as a food are associated with its mechanical action, and when by reason of such action conditions in the alimentary tube are raised from sub-optimum to optimum level, bran shows a nutritive value much above that of its comparable net energy value.

An interesting example of the necessity for bearing in mind the mechanical aspect of a dietary arises out of the interpretation of the results obtained by Crampton (36) in the feeding of a protein supplement containing 40 per cent. tankage, 20 fish meal, 20 linseed meal, and 20 minerals. When this supplement is fed to the extent of 30 per cent. the entire ration contains 6 per cent. of linseed meal, which, as recognised by the devisor of the supplement, may beneficially influence the mechanical condition of the dietary.

A vigorous controversy has arisen over the food value of roots. From the mechanical point of view they are an extremely valuable food, but obviously excessive root feeding, by producing a condition akin to diarrhœa, is wasteful. It used to be said that the water of roots is particularly good water, but this was merely another way of expressing the idea that moderate root feeding made for optimum conditions in the food tube and consequently promoted thriftiness of the animal. Much of the merit of pasture herbage and other green feeding is explainable similarly.

The value of green herbage for the pig is the subject of much controversy. The writer (12) found that the value of a separated milk meal ration is not enhanced by green feeding. Davidson (13) showed that "when a ration is properly balanced the addition of green food makes little if any difference," and examination of Davidson's data shows that the use of the word "balanced" referred to the "bulk" or perhaps more comprehensively to the physical condition of the diet. Yet Fishwick (14) claims a special value in pasture for pigs, especially for backward animals, pigs on pasture showing better health, less digestive trouble, and better appetite than similar animals confined to the house. The experiments of Dunlop (15) would suggest that the explanation of Fishwick's results lay in the low vitamin A content of his indoor rations, vitamin A deficiency having been shown by Hughes (16) to adversely affect rate of growth in pigs after a feeding period of six months. Hughes and Hart (17) present evidence which suggests that the vitamin A contained in the forage was a factor responsible for the greater gains by those animals which had access to pasture. Crowther (18) in discussing the question of pasturage for pigs wisely directs attention to the complexity of the problem. In order to

compare indoor and outdoor (on pasture) pig-feeding it is necessary to have regard to the physical character of the ration. From the results of a preliminary test—Experiment XIII—conducted by the writer in connection with this investigation it would appear as if this aspect of the problem were equally important with the supply of vitamin A.

Foods of a bulky character may be divided into two groups, namely, those which, like straw or dried grains, are bulky because of high fibre content of low digestibility, and those foods such as roots, young green herbage, and steeped bran which contain a very high proportion of water, but which are highly digestible. The large volume occupied by a unit weight of digestible material relegates both to the category of bulky food in so far as, in view of the limited capacity of the stomach, it is physically impossible for an animal to ingest a large quantity of nutriment in either form. Yet the mechanical effect on the large intestine of a dry fibrous food is very different from that of a succulent one. In the case of foods which already contain water or which absorb large quantities of water "bulk" affects not alone the stomach but also the large intestine. The presence of succulent food material in the lower gut maintains a high degree of liquidity therein, thereby maintaining the tube distended and providing a mild stimulus to peristalsis, and so causing the free passage of undigested material along the tube and aiding its ready evacuation.

Foods of this nature may be said to have a lubricative effect, but that this is due to their great affinity for water is shown by the bulky nature and high water content of the fæces. A truer lubricative property is exhibited by certain foods of which flax seed is an instance. In this case the mucilaginous coating of the seed on the absorption of a small quantity of water forms a gelatinous mass which is poorly digestible, and which, passing along the canal, acts as a lubricating medium expediting the movement of the contents of the canal, especially in the colon and rectum. Here again, however, the absorption and retention of water are operating factors, but lubrication of the tube may be effected without necessarily presupposing such action, because the more rapid movement of the lubricated contents along the lower gut would cause the evacuation of softer and more bulky fæces than would be the case did stagnation of the gut and excessive drying of the fæcal residue obtain. Liquid paraffin, for instance, lubricates the tube and appears to act merely as a preventive to the clogging of the lower gut and the accumulation there, for an abnormally long period, of fæcal residue. Most foods of high oil content, such, for instance, as rice meal, also possess a laxative tendency.

Because certain bulky succulent foods as well as some non-bulky foods have a specific effect on intestinal and particularly rectal activity, it would make for clarity of thought in relation to feeding if the term "bulk" in reference to foods were used only in relation to the filling of the animal's stomach, and the mechanical effect of foods on the intestine separately considered.

The new conception of "bulk" put forward by Proctor and Wright (19) contributed towards a better understanding of this feature of nutrition, but it

also indicated its complexity. These authors show, for instance, that, on soaking, decorticated cotton seed cake swells about twice as much as maize meal or wheat sharps. Yet it cannot be claimed that cotton cake is twice as bulky, using bulk in the sense in which it is usually understood, as either of these other foods. Conversely, according to their figures, maize meal swells to as great an extent as brewers' grains, but while maize meal is a concentrated food the grains are bulky. Again, the remarkable increase on soaking of coco-nut and linseed cake would, if this were adopted as a criterion of bulk, render these foods very much more bulky than most other cakes or meals, an idea which is in conflict with the general conception of their comparable value.

Reference to "bulk" in poultry feeding is made by Habe *et al.* (20), Halnan (21), and also by Shaw and Fisher (22). The last mentioned show that mashes made bulky by the use of bran give excellent results in practice, and they further point out that in the matter of "bulk" what matters is not so much the "bulk" of any particular unit of the mash as that of the food mixture fed.

Any excess of fibre or "bulk" in a ration obviously acts as a limiting factor to the intake of nutriment and consequently to the animal's progress. Similarly a deficiency may limit the nutritive value of the ration, so that the addition of a bulky food ingredient may by its beneficial mechanical effect compensate for the reduction in the energy value of the mixture.

When the addition of a fibrous food like oats to a concentrated ration, notwithstanding the reduction in energy value of the feed, causes no reduction in its nutritive value to the animal, and when the fibre actually confers a benefit by its presence, the beneficial effect would appear to consist partly at any rate in the opening of the stomach contents to the action of the gastric juice. The digestive juices are thus enabled to establish contact with the food particles more readily, gastric digestion is made more rapid, and the food leaves the stomach sooner than it otherwise would. The same object is achieved by giving a concentrated food in a flaky preparation rather than as a fine meal. Thus Woodman (23) found that when flaked maize and maize meal were fed as the sole diet the former was appreciably more digestible. This result is no doubt explainable by the superior mechanical condition of the flaked product, because other experiments have demonstrated no improvement in a cereal by the cooking process. When cooked flaked maize and maize meal are compared with one another in a ration which is otherwise very concentrated the former gives better results, yet when Crowther (24) combined each to the extent of 30 per cent. with barley, sharps, tapioca, soya bean, and minerals and compared the rations he found that, beyond a somewhat higher palatability of the mixture containing the maize flakes, the results, both in the way of live-weight gain, and of meal consumption per pound of gain, were similar. In the case of this experiment it would appear as if the remainder of the ration was such that when the maize flakes were added to it the resulting pig ration was not superior from the mechanical point of view to the comparable ration containing maize meal. There is a further possibility that in the case of this experiment the flakes did effect some mechanical improvement in the ration, but that the expression of

this improvement was prevented or rather offset by excessive "bulk" in the flake mixture.

From the practical aspect foods must be so combined as to provide a mechanically suitable mixture. V. E. (25) advocates, so as to prevent digestive disturbances and to promote active digestion, the mixing of wheat, rye, and maize, which are low in fibre, with foods such as oats, which are rich in fibre. Wright (26) found that when the barley and tapioca in a diet consisting of barley, tapioca, sharps, soya bean, and minerals were replaced by potatoes to the extent of 22 per cent. no change was effected in the rate of progress, on the economy of food consumption, or on the "bloom" of the pigs, but that at 66 per cent. replacement was detrimental in all these respects. That a two-third replacement of the meals by potatoes was reflected in a lesser "bloom" of the skin is also reported by Wallace and Thompson (27). Experiments with fattening cattle conducted at the Purdue University (28) showed that when in a standard ration of corn, cotton seed meal, hay, and silage one-third of the corn was replaced by oats, whose fibre content was higher, greater economy of food consumption was obtained. Total replacement of the corn by oats reduced the efficiency of the ration. Gartner and Gaede (29) state in connection with the rations they employed in pig-feeding that supplements of higher fibre content and lower digestibility can be used without reducing gains in weight or feed utilisation. In experimental work on the utility of dried sugar beet pulp Woodman, Duckham, and French (30) found that while swine digest this food almost equally well with ruminants, the great bulk of the pulp rendered it unsuitable except in small quantity for pig-feeding, and Crowther (31) found that it was necessary to feed pigs three times daily in order to enable them to utilise satisfactorily a ration containing 20 to 25 per cent. of beet pulp. The utility of a considerable amount of bulk and fibre in the ration of poultry is well recognised. Fangauf (32) claims that, in the case of fowl, crude fibre in large quantity does not hamper production. Results obtained at the Oklahoma Agricultural Experiment Station (33) show that even in the case of turkey poults rations moderately high in fibre were superior to those of low fibre content.

The question of the maintenance of intestinal activity is closely associated with good health and general body well-being. Bran, linseed cake, coco-nut cake, roots, and green herbage are instances of foods which promote healthy activity of the alimentary canal. The fæces evacuated by animals so fed are comparatively bulky, and of a soft consistency, and they appear to contain a larger proportion of the digestible food ingested than do fæces from animals fed on concentrated foods. The following are rough determinations of the total daily weight of fresh and of dried fæces from two similar groups of 8 pigs eating similar quantities of food and fed on different diets :—

	Fresh fæces.	Dried fæces.
Diet A—concentrated diet	47 lb.	9·4 lb.
Diet B—diet containing 20 per cent. of bran ...	63 lb.	11·2 lb.

Yet the animals on diet B were making as much progress on the basis of live-weight increment as those on A, and they showed better health and more "bloom." It is, of course, clear that the voiding of excessively soft fæces is wasteful, an extreme case of which occurs in diarrhœa. But a certain amount of liquidity, involving an obvious loss of nutriments, appears to be necessary for the maintenance of optimum mechanical condition in the gut. The normal stimulus to peristalsis is the dilatation of the wall by the contents, and a gut dilated with fairly liquid contents functions better than one which, because of excessive absorption of liquid, is collapsed. The special physical merit of bran, green food, linseed cake, green feeding, and roots appears to lie in their ability to produce such conditions in the lower gut. The mucilage of flax seed, the pectins of roots and fruits, the galactans of seaweeds, and to some small extent of certain cereals, and the gums of fruits are only slightly acted on by the digestive juices, and hence they carry their water-holding properties right through the tube. The fibre of bran appears to possess similar properties.

Animals on a dietary which, by inclusion of some of the foods just referred to, is mechanically suitable develop a sleek coat and clean healthy skin: they exhibit the quality known as "bloom," which appears to be nothing more than an outward indication of the operation of optimum mechanical conditions throughout the alimentary tube. The absence of such conditions operates more severely on young than on older animals, and, while certain strong members of a group may be able to tolerate adverse conditions, the smaller and younger members succumb, and hence the remarkable unevenness of pigs fed a physically unsuitable dietary. Such want of uniformity was observable in some of the experiments reported herein. Crichton *et al.* (34), when comparing potatoes with maize, also noted that while the pigs on the potato group grew evenly the maize group was quite uneven. The writer observed that when a mechanically unsuitable diet is rectified the desire of pigs to chew at foreign substances and the spirit of restlessness tends to disappear. Similarly in the case of chickens: feather picking was less prevalent on a mechanically suitable diet containing a high proportion of bran than on a very concentrated one. The substitution of oats for bran in the chickens' diet was not, however, from this point of view, satisfactory.

In view of the difference, as illustrated by the effect on the animal, in the quality of fibre from different sources, and because of the presence in many foodstuffs of ingredients which have a specific mechanical effect on the alimentary tube, it is difficult, if not impossible, to define with exactitude the optimum percentage of fibre in a ration. An approximation can, however, be made, and the results of the experiments reported go to show that the optimum amount of fibre in the ration of a pig lies between $3\frac{3}{4}$ and $5\frac{1}{2}$ per cent. A statement of the exact proportion of fibre which would give best results is possible only in reference to a particular type of ration, the ingredients of which are known.

Penquite and Thompson (35) secured better results as regards rate of

growth and mortality among chicks from the use of fibre from alfalfa stems than from the fibre of cotton-seed hulls. Dehulled oats (groats) are held in high esteem for young stock; and Crampton (36) showed that, when added to a protein supplement containing little or no fibre, ground oat groats gave superior results to maize meal in pig experiments; and Carroll and Carrigus (37) showed that a mixture of dehulled oats and maize was superior to maize. The dissimilarity of the results from these two foods of similar fibre content may be explained by the more favourable mechanical effect of the groats. In experiments conducted by the writer concentrated rations containing a moderate proportion of groats (2 per cent. of fibre) did not prove decidedly costive, due apparently to a certain lubricative effect on the gut exercised by the groats.

Foods whose mechanical effect is akin to that of roots stimulate appetite. In the experiments reported herein the inclusion of bran or linseed cake in the ration caused an increase in food consumption as compared with the standard rations. Weber (38) reported that young cattle receiving linseed meal as a supplement made larger gains, but ate more than those getting cotton-seed meal or gluten meal. Crowther (39), on comparing sharps with oats in a diet otherwise consisting of barley, tapioca, maize, and soya bean, and working with pigs of 38 pounds initial weight, reports greater food consumption and higher gains by the sharps group for the first 112 days of the test, but a similar food consumption and similar live-weight increase thereafter. Presumably, the pollard made for more favourable conditions in the tube of the young pig than did the oats. Practical feeders are familiar with the appetising effect of a proportion of turnips in the ration of fattening animals. Appetite is a phenomenon which is associated with the tone of the stomach wall, but this again is intimately connected with the condition of the colon and rectum. An extreme illustration is where constipation causes complete loss of appetite and induces vomiting. It would appear, therefore, that the increased appetite on diets containing such foods as linseed bran and roots must be attributed to the improved functioning of the large intestine, due to the superior mechanical condition of diets so constituted.

A fuller recognition of the importance of the mechanical aspect of nutrition would help to harmonise the outlook and views of the pure scientist and the practical man on matters of the everyday nutrition of farm stock. Thus, bran not alone contains so many calories of digestible energy, but its flaky form and moderate percentage of soft, highly absorptive fibre give it a value for stock feeding which is higher than is represented by its comparable energy value. The expression of the special virtues of bran depends, however, on the choice of other materials which with bran constitute the ration. The popularity of crushed oats as a food for cattle, sheep, and horses is largely due to its flaky character and to its fibre content, which appears to be approximately optimum in quantity for the animals mentioned. Linseed cake is a concentrated, but yet not costive, food, for which reason it is superior in certain circumstances to other foods of similar net energy value. Like linseed cake, carrots maintain

the alimentary canal of an animal in a particularly healthy condition, effecting regular evacuation, but yet not unduly accelerating peristalsis. Probably the favourable mechanical effect of carrots is due to their high content of pectin, which from the chemical point of view is only valued as a simple carbohydrate. Nor can roots of any kind be valued solely on their chemical composition. It is well known that a small quantity of roots enhance under many circumstances the value of the ration. As a food, they give increasing return up to a certain point, i.e., a certain daily consumption, above which the return per unit fed progressively declines.

Each particular food possesses physical attributes peculiar to itself, and it is the expression of the sum total of these in a ration which determines the mechanical effect. A "heating" effect is said to be produced by a particular dietary, which when examined is found to be predominantly carbohydrate in character, and to be of a particularly concentrated nature. The "heating," in view of the composition of the mixture, could in such cases have no connection with the phenomenon of "specific dynamic action," and is possibly attributable to a slight fever due to sluggishness of the lower gut, and the consequent continuous absorption of injurious fermentation products which, were peristalsis more rapid, would either be evacuated with the fæces or perhaps not produced at all.

It is clear from the data presented that in the determination of true nutritive value the food being tested should be incorporated into a ration, the remainder of which, from the point of view of its mechanical effect inside the food tube, is suitable. If the added food effects an appreciable improvement in the mechanical condition of the complete ration it may be credited with a higher value than its chemical constitution warrants. In addition to their chemical value so obtained certain foods have specific physical attributes of very definite utility to the animal, but unless the chemical and physical characters are differentiated, unanimity in the results from different investigators and from repeated experiments on the nutritive value of individual foods cannot be obtained.

Without distinguishing between the mechanical and the chemical aspects of the question Forbes has drawn attention to the necessity for evaluating a food not as a separate entity, but as a part of a complete ration, and in this connection he has formulated what he describes as "The Law of Maximum Nutritive Value" (40), which reads as follows: "An individual foodstuff expresses its normal and most characteristic nutritive value, for a given kind of animal, under specified conditions governing nutritive requirements, only as it is a part of a ration which is qualitatively complete and quantitatively sufficient, for the conditions existing . . ." In association with colleagues, Forbes (41) found, for instance, that the net energy of corn meal was greater when fed in conjunction with alfalfa than when fed alone; and, again, the same authors (42) found when maize was added to three different types of ration that "the apparent digestibility of the corn meal differed decidedly as affected by the

other constituent feeding stuffs of the rations in which it was fed and therefore affected the net energy values determined." Foodstuffs express their full nutritive value only when they constitute part of a nutritively complete ration, but the conception of nutritive completeness must be extended beyond the chemical aspect. A chemically complete ration may yet be physically unsuitable, so that a food included in a mixture containing all the known essential nutritive ingredients may not express its full nutritive value unless the mixture of foods in the ration is such as to promote optimum mechanical conditions in the alimentary tube. Rations must be balanced not alone in respect of chemical constituents, but also with regard to physical constitution.

An incorrect physical condition of a ration is very readily reflected in the "form" and health of the animal. The maintenance of optimum mechanical conditions in the food tube is reflected not alone in general vivacity and health, but in the external appearance of the skin. The physical character of the ration affects the size and density of the fæces, and conversely the appearance of the fæces may be used as an indication of the suitability or otherwise of the ration from the mechanical point of view. Any disorder of the food tube, and a variation from the optimum mechanical condition may be considered a disorder, is reflected in the animal's progress, so that the daily gain of the beast, if it is a fattening animal, or the daily output of milk, if a milking animal, is a reflection of the mechanical fitness of the dietary. The cumulative effect of unsuitability in this direction becomes apparent, for instance, in a group of young fattening pigs in the development of non-uniformity among the animals as compared with the uniformity of a similar group of animals on a satisfactory ration.

The feeding, during the early growing stage, of a very concentrated ration appears to produce a permanently detrimental effect. Animals so reared are unable, in later life, to utilise such large quantities of food, particularly bulky foods, as individuals reared on bulky diets. The other extreme is the case of pot belly, due to excess of bulk and insufficiency of nutriment. Hence the particular necessity for the choice of suitable dietary in early life. The skilled practical feeder bears this in mind, particularly in the case of pigs and dairy heifers. Possibly the better physique and greater vigour and higher resistance to disease of the human who is fed brown bread rather than white in early life is explainable to a large extent by the superiority of the coarser and more bulky and branny brown bread from the mechanical point of view. It is possible that the good results obtained from the feeding of brown bread rather than white to racing dogs are similarly explainable.

The type of animal fed and the kind of work it has got to do must be borne in mind in considering the physical condition of a ration in its relation to nutritive value. The pig has a greater tolerance than other species for concentrated foods, especially those of a doughy consistency. Materials of this type in the case of the sheep readily produce digestive troubles, and often cause fatalities. Intelligent shepherds, feeding, for instance, maize and barley,

like to mix therewith an equal weight of such materials as dried grains and bran. Perhaps the animal which most readily responds to alteration of the physical character of the diet is the horse, in which case choice of food for animals doing different types of work is certainly as much a matter of the mechanical as of the chemical aspect of nutrition.

The combinations of the variety of available foodstuffs which, for each type of animal, make for optimum conditions in the stomach and intestines is a subject worthy of careful scientific study.

Summary.

The addition of linseed cake meal to a concentrated ration increased the appetite of pigs and enhanced the value of the ration: when the ration included 25 per cent. of bran no beneficial effect from the addition of linseed cake meal was observed. In the former case the effect was correlated with a change in the character of the fæces, particularly in the smaller and younger pigs, and with greater uniformity of growth among the individual animals in the group.

Oats, with a fibre content approximating to that of linseed cake meal, did not exert a like favourable influence on a concentrated ration.

A concentrated and costive ration, composed of maize, barley, meat meal, and salt, and which caused rapid fattening with little growth, was improved by the addition of bran. A keener appetite, better health, more growth, and fæces of greater bulk and softer consistency marked the change. The optimum proportion of bran in this mixture seemed to lie between 10 and 20 per cent.

The addition of carrageen moss and of liquid paraffin to a very concentrated diet caused pigs to be less restive and to appear more satisfied with their conditions in the sty than comparable animals on the normal ration not so supplemented. Judging from the change in the character of the fæces, the action of the carrageen moss and liquid paraffin was located in the lower gut, where, temporarily at any rate, these dietary supplements promoted more regular evacuation and the voiding of softer fæces.

The nutritive value of a concentrated meal ration as judged by live-weight increase of pigs was not reduced by the addition thereto of oats up to 30 per cent. of the mixture. Apparently the improvement in the physical character of the ration effected by the addition of the oats compensated for the reduction in the net energy value of the mixture. Because the fæces of the oat-fed pigs were not appreciably larger or softer than those of comrades on the concentrated ration, and because also the oat-fed pigs lacked the "bloom" which is associated with foods that exercise a beneficial mechanical influence on the lower gut, it would appear as if the ameliorative mechanical effect of the oats operated principally in the stomach. The addition of oats to a ration which already contains some bran or linseed cake did not effect any improvement from the physical point of view. Under the latter conditions oats gave a result definitely inferior to maize, while under the circumstances of the former experiment oats and maize had a similar nutritive value.

Neither the rate of gain nor the economy of food consumption was improved by the addition of linseed oil to a low oil concentrated meal mixture.

Experimental data showed that the particular merit of green feeding in the dietary of the pig was due both to its vitamin A content and to its beneficial mechanical effect.

The fibre of bran is superior to that of oats: the superiority is discussed in relation to the functional activity of the large intestine.

Apparently the presence of a certain amount of mucilaginous material explains the special merit of linseed cake, the specific nutritive value of which is discussed.

The preparation of a foodstuff in the flaked, as distinct from the meal, form confers on it, as far as gastric digestion is concerned, an advantage similar to that of adding fibre to a concentrated ration, but a diet excessively rich in flaky ingredients may prove so bulky as to limit the ingestion of nutriment.

Bulky foods belong to two categories, namely (*a*) that to which straw and dried grains belong, and (*b*) the group which includes succulent and lubricative foods such as roots, green food, steeped bran. The mechanical effect of the former is chiefly concerned with stomach activity, while that of the latter is felt as much in the large intestine as in the stomach.

The influence of succulent and lubricative foods on the lower gut is discussed.

It is suggested that it would make for clarity of thought if the effect of "bulk" was considered only in relation to stomach function and the mechanical action of foods on intestinal, especially rectal, activity was not confused therewith.

Excess of "bulk," whether the bulky condition is due to a high fibre or water content or to the preparation of concentrated foods in a flake form, acts as a limiting factor to ingestion of nutriment and to production: conversely the addition of a bulky food constituent may so improve the physical condition of a ration as to compensate for the reduction in the net energy value of the mixture.

The smooth functioning of the lower gut is necessary for the promotion of good health and general well-being. There is in that organ an optimum condition of affairs which lies between the two extremes of constipation and diarrhœa, and attention is drawn to the importance of those foodstuffs which promote such optimum condition even at the sacrifice of a certain amount of digestible material in the fæces; the dilation of the lower gut by moderately liquid contents is important.

The "heating" effect of certain foods is discussed: the phenomenon is probably due to sluggishness of the lower gut.

A physically unsuitable dietary operates more adversely on young than on older animals, and there is evidence that the rearing of an animal on an excessively concentrated diet produces a permanent disability. Likewise an excessively bulky diet fed over a prolonged period in early life may permanently

injure the system, such as occurs when the condition known as "pot belly" is produced.

The physical condition of a ration is reflected in the character of the fæces, in the progress or production of the animal, in its state of health and vigour, and in the appearance of the skin.

On the assumption of the dietary being complete from the point of view of chemical constituents, "bloom" is interpreted as an external indication of the existence of optimum mechanical conditions in the alimentary canal.

Foods such as bran, linseed cake, fresh green herbage, carrots, coco-nut cake, etc., may have in certain circumstances a nutritive value greater than that represented by their net or available energy content.

Evidence is presented showing the necessity, in comparing the nutritive value of foods, of having regard to the mechanical aspect of nutrition: neglect of this may lead to incorrect conclusions. In determining the true or relative value it is necessary to incorporate the particular food under consideration into a ration which is otherwise at the optimum degree of mechanical suitability.

Attention is drawn to the necessity in practical feeding for combining foodstuffs so as to provide a mechanically suitable mixture.

The connection between the physical character of a diet and its nutritive value should be considered in relationship to the type of animal fed.

Because of the different effect, in the alimentary canal, of fibre from various sources, and in view of the specific mechanical properties of many of the foodstuffs which are included in the dietary of farm animals, it is difficult to specify exactly the optimum percentage of fibre in a ration, except when the foods which enter into the ration are known.

The varying degree of appetite provoked by different foods in a dietary mixture is correlated with their mechanical effect on the large intestine.

A fuller recognition of the importance of the mechanical aspect of nutrition would help to harmonise the outlook and views of the scientist with those of the practical feeder on matters of the every-day nutrition of farm stock.

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No. 30.

THE NITRO-CHROMIC REACTION AND ITS APPLICATION TO THE ESTIMATION OF SMALL QUANTITIES OF ALCOHOL.

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[Read 17 December, 1935. Published separately 28 January, 1936.]

The use of a solution of potassium dichromate in nitric acid for the detection of alcohols was first described by Agulhon (*Bull. Soc. Chim.*, 1911, **9**, 881). According to him the reaction, which consists in the reduction of the dichromate so as to give a clear blue colour, is also given by aliphatic aldehydes and ketones. Fearon and Mitchell (*Analyst*, 1932, **57**, 372), using a modified form of the reagent, found that under controlled conditions it could be made to react positively with primary and secondary alcohols, hydroxy-acids, sugars, and formaldehyde, but negatively with other aldehydes, tertiary alcohols, ketones, and polysaccharides. Craven (*J. Soc. Chem. Ind.*, 1933, **52**, 239 T) found that the test could be applied quantitatively to the analysis of industrial solvents, e.g., in estimating alcohol in acetone, or formic acid in acetic acid. He used Agulhon's reagent almost unmodified, and in all his estimations the end-point was defined as the attainment of a pure blue colour within a given time (five minutes).

In the course of an investigation into the possible clinical and biochemical uses of the test, as applied to ethyl alcohol, it became apparent that in the interests of greater sensitivity and specificity certain modifications of the original technique were desirable. These include a decrease in the concentration of both nitric and chromic acids, and an extension of the period of the reaction.

Technique.

Conditions for Maximal Sensitivity.—It was found by experiment that dilution of the nitric acid, while in no way impairing the sensitivity of the reagent to reduction by alcohol, and only slightly increasing the reaction time, had the advantage of rendering it less reactive towards other substances, and in general easier to work with. A concentration of one part of strong nitric acid to two of water was used throughout, but the precise strength does not seem to be of any importance. On the other hand, too great a concentration of chromic acid renders the reduction of a fraction of it impossible to detect,

as the orange colour is much deeper than the blue, and masks it. For most purposes, a mixture containing one part of 5 % potassium chromate solution in 100 parts of diluted nitric acid was proved to be satisfactory. Such a solution contains 0·258 g. CrO_3 per litre.

If to 10 c.c. of this solution (hereinafter called simply nitro-chromic acid) be added 0·8 c.c. of a 0·1 % solution of alcohol the orange colour is eventually completely discharged and replaced by blue. This corresponds to an alcohol concentration of less than 0·008 %. Considerably smaller quantities can, of course, be detected by a less complete change of colour, and roughly estimated colorimetrically. As Craven has already pointed out, the colour of the solution is still yellow even when 90 % of the chromic acid has been reduced, so that it is hard to make such colorimetric determinations at all accurate; nevertheless, it is considerably easier in the weaker solution than in Agulhon's original reagent. For accurate work it is essential either to use varying dilutions until the amount of solution that just causes the appearance of the pure blue colour is ascertained, or else to add between 90 % and 100 % of the required quantity of alcohol, thus producing a green shade. Over this part of the range the change in colour is sufficiently rapid to reduce the error to a maximum of 2 % when the colour is matched against a series of standards.

For still greater dilutions the CrO_3 content of the reagent may be reduced to a fifth of that specified above. At this strength an alcohol concentration of 0·001 % is sufficient to reduce the chromic acid completely (though the blue colour produced is so faint as to be almost imperceptible), and a concentration as low as 0·00025 % produces a perfectly definite colour change. The increase in sensitivity on dilution is to some extent compensated for by the fact that the initial colour of the reagent when diluted appears greenish yellow rather than clear orange.

Time and Temperature of the Reaction.—As in most other chemical methods speed and accuracy are inversely proportional to one another. Unless quick results are a primary consideration, it is far better to let the reaction complete itself than to judge it by the colour attained after five minutes. For one thing, this extends the usefulness of the method to low concentrations that would otherwise give a negative result. At considerable dilutions the speed of reaction is, of course, slowed up, and only a very small fraction of the alcohol is oxidised in the first five minutes. Furthermore, for accurate results the timed reaction would have to be carried out at a standard temperature (which has not yet been specified), since the difference between the summer and winter temperatures of an ill-heated laboratory is enough to cause a serious error. At ordinary room temperature the time required to reach equilibrium varies from an hour to over twelve hours, according to the concentration; but the tubes may be kept at 50° C. without sensibly altering the end-point, and by this means the reaction reduced to a maximum of two hours. Nevertheless, for really accurate work it is probably safest to leave them overnight. There are some cases, of course, in which the reaction has to be limited to a definite time,

e.g., in the presence of acetone, which is eventually completely oxidised, but at a very much slower rate than alcohol. In such cases a series of empirical standards, tested at the working temperature, must be used.

Methods.—For the analysis of aqueous solutions of alcohol which contain no other solute likely to react with the nitro-chromic acid, the easiest method is to run 10 c.c. of the latter into a test-tube, and add varying amounts of the alcoholic solution until the approximate end-point is determined. For adding small quantities it is a convenience to have this solution contained in a burette which delivers accurately 20 drops per c.c. The tubes are then matched against a previously prepared series of standards. These may be made by adding to 10 c.c. of nitro-chromic acid 0, 0·1, 0·2, 0·3, 0·4, 0·5, 0·6, 0·65, 0·7, 0·75, and 0·8 mg. of alcohol. As has already been pointed out, the matching may be most accurately carried out over the range of greens. For the analysis of very weak solutions which it is undesirable to dilute further, it is convenient to have a solution of nitro-chromic acid three times the standard strength, i.e., 0·15 % K_2CrO_4 in pure nitric acid. 5 c.c. of this are run into 10 c.c. of the alcoholic solution.

There are many cases where the use of a Conway unit is desirable, instead of actually mixing the reagents. This is a circular dish, divided by a ridge into an outer annular and an inner circular compartment, the whole being sealed up by a ground-glass plate. 2 c.c. of nitro-chromic acid are put in the central compartment, and 2 c.c. of the alcoholic solution, diluted if necessary, in the outer. There is a progressive distillation of alcohol into the inner compartment, where it is "fixed" by reacting with the nitro-chromic acid. To ensure a complete distillation it requires at room temperature at least 24 hours, but in many cases the unit may with safety be kept at 50°. The advantages of this method are numerous. It can be employed when only a very small quantity of the fluid is available in such cases as blood serum, for it is possible to detect the alcohol in 0·1 c.c. of a 0·025 % solution. It is useful for strongly coloured fluids and for those containing non-volatile solutes liable to be oxidised by the nitro-chromic acid, such as sugars.

Applications.

1. *The Determination of Alcohol in Expired Air.*—The method adopted for obtaining approximate results was to put 50 c.c. of nitro-chromic acid in a glass cylinder, and to expire continuously for five minutes through a tube which passed through the rubber bung in the mouth of the cylinder and ended in a Folin absorption bulb below the surface of the liquid. After a further ten minutes the colour of the liquid was examined. Under these conditions about 15 mg. of expired alcohol vapour is sufficient to reduce the chromic acid completely, and about 4 mg. to produce an easily perceptible colour change. That absorption by the nitro-chromic acid is practically complete was proved

by passing the air issuing from it into a second cylinder containing iodine and KOH.

2. *Applications to Biological Fluids.*—By means of the Conway unit alcohol may readily be determined in such fluids as milk, blood serum, saliva, and urine. If possible, these should be left for 48 hours at room temperature, since warming accentuates the tendency of the nitric acid vapour to attack other organic constituents and liberate volatile substances, which reduce the nitro-chromic acid. In the case of urine there is, owing to this, a small zero error even in the cold, equivalent to about 0·0015 % of alcohol, but it can be allowed for by setting up a control, and the error is not very serious. It is, of course, unsatisfactory to treat urine directly with the nitro-chromic acid, both on account of these subsidiary reactions, and also owing to the brown colour produced by the action of nitric acid on the urinary pigments.

3. *Estimation of Alcohol in Industrial Products.*—Craven has already given details for acetone, which is perhaps the most important industrial application; but the method may also be extended to fluids, such as petrol and ether, that are immiscible with water and liable to contain alcohol. They are first shaken up with water, and the aqueous extract then analysed directly. The partition coefficient of alcohol between the solvent and water must, of course, be determined first.

No. 31.

THE PREPARATION OF ALGINIC ACID AND ITS CONSTITUTION.

By VINCENT C. BARRY, M.Sc.,
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[Read February 4. Published separately March 20, 1936.]

At the end of 1934 we described briefly, in a preliminary note (1), a new method for the preparation of alginic acid from seaweeds. We now propose to give a detailed account of this process, which greatly simplifies the extraction of the body, and gives a purer product than any of the older and more laborious methods.

Our experiments have so far been conducted with the fronds of *laminaria digitata*. To obtain the alginic acid, the fronds are covered with water in a beaker, and boiled for 15 minutes; the water is decanted off, and the same process is repeated twice. The material left after the third boiling is covered with a mixture of equal volumes of water and concentrated hydrochloric acid, and left standing for 24 to 48 hours, after which the acid is poured off, and the contents of the beaker are washed with water. Five per cent. ammonia is now added, and, when the beaker has stood on the water bath for fifteen minutes, the ammoniacal extract is poured off. This extraction is repeated twice, and the three ammoniacal extracts are mixed together and filtered through filter-paper, preferably without pressure. Whereas solutions of alkali alginates prepared by the usual methods will scarcely pass through a filter-paper at all, the above extract will pass through completely in a few hours. From the filtered extract concentrated hydrochloric acid precipitates the free alginic acid as an almost white jelly. This is filtered off through a glass filter and bleached by mixing with chlorine water on the filter. The chlorine having been washed off with warm water, the jelly is boiled with spirit, and, the spirit having been sucked off at the pump, the acid is redissolved in ammonia. This ammoniacal solution is filtered through paper as before, and gives a clear, almost colourless, filtrate. The alginic acid having been reprecipitated from this filtrate with hydrochloric acid, is filtered through a glass filter, washed with water, and boiled alternately with water and spirit twice. Finally, if it is desired to obtain the dry acid, it is boiled with absolute alcohol, filtered, washed with ether, and dried in the steam oven.

Alginic acid prepared in this way is a white powder, free from chlorine and from all but traces of ash. We determined its molecular weight by the analysis of its barium salt, which was prepared in the manner described in a previous communication (2). For the analysis this salt was dried in a vacuum of 0·02–0·05 mm. at a temperature of 70°–100° C. until the weight was constant. A weighed quantity was then burned, and the ash converted into barium sulphate. The following results were obtained:—

I. 0·1040 g. gave 0·0470 g. of barium sulphate. Equivalent weight of free alginic acid, 190·5.

II. 0·1021 g. gave 0·0455 g. of barium sulphate. Equivalent weight of alginic acid, 194·2. ($C_6H_{10}O_7 = 194$.)

The specific rotatory power of the sodium salt was determined from a solution made by titrating the gelatinous acid with $N/10$ caustic soda and making up the resulting solution to 100 c.c. The quantity of sodium alginate in the solution was calculated on the assumption that the formula was $C_6H_9O_7Na$. The following results were obtained:—

	I.	II.	III.
Volume of caustic soda in c.c. ...	31·84	36·98	38·18
Rotation	−1·825	−2·133	−2·178
$[\alpha]_D^{10}$ for sodium alginate calculated	−132·4°	−133·4°	−132°

Gomez (3) gives a list of 12 determinations of the specific rotatory power of sodium alginate. Nine of these lie between 130 and 137, and the mean of these is 133·17, in good agreement with the mean—132·6—of our three results.

Most of the experimenters mentioned by Gomez give no account of the method by which they obtained a sodium alginate solution of known strength. It is certain, however, that none of them found the strength of the solution by a calculation such as ours, based on an assumed formula for the acid. The agreement between our results given above and the other determinations shows, therefore, that sodium alginate can be represented by the formula $C_6H_9O_7Na$.

The results of polarimetric measurements and analyses here recorded support the view, already put forward and made the basis of a tentative constitutional formula (2), that the polymerising unit in alginic acid is not an anhydride, but the complete uronic acid.[1] Several analyses by other workers (3, 4, 5) also point in the same direction.

[1] Two years later Gomez (3) put forward the same constitutional formula as that given by Dillon and McGuiness, adding in a footnote that this was the formula that they intended to give, but that "in their work it does not appear so" for the reason that two hydrogen atoms, which have nothing to do with the polymerisation, are omitted in error from one of the uronic acid units shown.

There are other possible explanations of the equivalent of alginic acid, as determined above, besides the assumption that the polymerisation has taken place without loss of water. For example, a substance with the formula $(C_6H_8O_6)_9(C_6H_{10}O_5)$ would have an equivalent of 194, and a percentage of carbon and hydrogen differing from that of $C_6H_8O_6$ by an amount inside the limits of experimental error. Such a formula would reconcile the equivalent of 194 with the results of the carbon and hydrogen determinations of Nelson and Cretcher and of Dillon and McGuiness. It would not, however, fit in with the equivalent of 179 found by Nelson and Cretcher for the dried acid by direct titration (2).

While the data with regard to alginic acid so far obtained indicate that the polymerising unit is the complete sugar acid, it must be admitted that the information available is not as yet sufficient to enable us to arrive at a definite conclusion. In dealing with a colloidal substance such as this, one can never be sure, even after drying in a high vacuum, to what extent water may still be adsorbed. Exact information as to the nature of the polymerising unit and the constitution of the molecule can only be obtained by a careful study of its derivatives.

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No. 32.

DERIVATIVES OF ALGINIC ACID.

Part I.

THE ACETYLATION OF ALGINIC ACID.

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[Read February 4. Published separately March 20, 1936.]

Derivatives of alginic acid of the ether or of the ester type are extremely difficult to obtain; a fact which perhaps accounts for the absence of any reference to such compounds in the literature. Gomez (1), it is true, asserts that he acetylated the substance with acetic anhydride and sodium acetate; but his products do not correspond in acid or in acetyl number to any definite acetyl derivative, and he isolated no body soluble in organic solvents. From our experiments it seems likely that he did achieve a small amount of acetylation; but his compound was mixed with a large quantity of unchanged alginic acid. Apart from this, no claim to the preparation of any derivatives of alginic acid other than salts appears to have ever been made.

We ourselves have, during the past few years, made repeated attempts to methylate alginic acid, without meeting with success. We have tried the action of methyl sulphate and alkali under various conditions, only to find that our original substance was either recovered unchanged or completely broken up.

Attempts to acetylate with the usual reagents were more successful than our efforts at methylation, but were still far from satisfactory. Acetic anhydride, in combination with pyridine or potassium carbonate, produced little or nothing soluble in organic solvents, nor did it make any change in the appearance of the alginic acid. With sodium acetate a substance soluble in acetone was obtained; but the fraction of the alginic acid affected was so small that the amount of soluble product available was only sufficient to obtain evidence of acetylation by micro-analytical methods. Sulphuric acid either produced complete acetolysis so that nothing was recovered when the temperature was allowed to rise, or left the alginic acid unaffected when the reaction mixture was cooled. Zinc chloride, on the other hand, gave a small yield of a substance soluble in chloroform, which seemed from a preliminary examination to be a triacetate of $(C_6H_{10}O_7)_n$.

Having failed to achieve any satisfactory results with the conventional reagents, we were obliged to try less common or entirely new catalysts with acetic anhydride. Sulphur dioxide and chlorine, which were first used for the acetylation of cellulose by Barnett (2), had no apparent effect on alginic acid. With iodine, which does not appear to have been used before, better results were obtained. Three successive treatments of a quantity of alginic acid with acetic anhydride and iodine converted it almost completely into an acetone-soluble product. This product was divided into two fractions. The neutralisation and acetyl values of one of these corresponded fairly well with a diacetyl derivative, and of the other with a triacetyl derivative of a uronic acid. The addition of sulphur dioxide did not improve the process in any way.

Since iodine had proved such a useful catalyst, it was decided to try whether hydriodic acid might not be still better. Here we met with a striking success. When alginic acid is well mixed with glacial acetic acid containing a little hydriodic acid, and warmed, it reacts rapidly with acetic anhydride on addition of that reagent. If, as sometimes happens, the reaction is delayed, it will start at once on addition of a trace of sulphuric acid. In either case almost the whole of the alginic acid eventually goes into solution. A similar pre-treatment with halogen acids is the subject of an English patent for the acetylation of cellulose (3).

The completeness of this reaction, and the yield and apparent purity of the products, led us to make a more detailed examination of the acetylated bodies formed, leaving the study of the reactions with zinc chloride and with iodine to a later date. The mechanism of the reaction is probably as follows :—

(1) $(CH_3CO)_2O + HI = CH_3COOH + CH_3COI.$
(2) $CH_3COI + HOR = CH_3COOR + HI.$

Its efficiency may be ascribed to the absence of water as a bye-product, and to the continuous reformation of acetyl iodide on the surface of the colloidal molecule.

When, at the end of the reaction, the liquid is poured into ether, the acetylated alginic acid is thrown down as a white flocculent precipitate, which can be easily freed from acetic anhydride by washing with ether. It amounts to about 60 per cent. of the alginic acid used. It can be divided into three parts by successive extractions with acetone, industrial alcohol, and aqueous alcohol. From 100 parts of acetylated product we obtain—

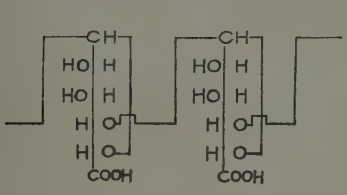
I. Soluble in acetone	18
II. Soluble in industrial spirit	32
III. Soluble in aqueous alcohol	42
IV. Insoluble residue	8

The three soluble bodies are all acetylated polymeric uronic acids. They are all colloidal in character, but differ in physical and chemical properties, in

appearance, in specific rotatory power, in their reducing action on Fehling's solution, and to some extent in the solubilities of their salts. However, neutralisation and saponification values show that the polymerising unit in all three has the formula $C_6H_8O_7(COCH_3)_2$. The percentage of carbon and hydrogen in one of them also agrees with this formula. In the other two the agreement is not so good; but it must be remembered that we are dealing with colloidal substances, the purification of which is extremely difficult. All three bodies appear to be acetylated degradation products of alginic acid of different degrees of polymerisation.

The three acetyl derivatives were saponified, and the degraded alginic acids were precipitated and analysed. The equivalents of all three corresponded well with that required by $C_6H_{10}O_7$. Their specific rotatory powers were all different, and were all less than that of alginic acid.

These facts have an important bearing on the constitution of alginic acid. It has already been suggested (4), on the basis of analyses of its salts, that the polymerising unit in the substance is not an anhydride (formula I), as in



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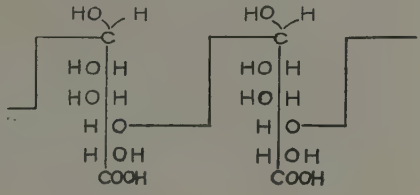
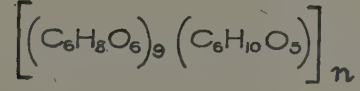


II



III

other polymeric carbohydrates, but the complete sugar acid (formula II). The possibility, however, that adsorbed water was the cause of this apparent anomaly of alginic acid could not be completely excluded. With the acetates the case is different. They have been prepared in conditions under which the presence of adsorbed water was, to say the least, extremely unlikely; yet they appear to be acetates of II.

Formula III has already been briefly discussed as another possible way of reconciling the equivalent with formula I (5). This association of one hexose anhydride unit with every nine uronic anhydride units would give an equivalent of 194, and have very nearly the same percentage composition

as I. The composition of the acetates excludes any such possibility. The carbon and hydrogen of an acetate of III would differ by more than 5 per cent. from the experimental results. Moreover, since our acetates are degradation products, if III were correct one might expect degradation to take place in such a way as to give compounds containing different proportions of hexose groups, and, therefore, having different percentage compositions and equivalents. The facts so far, therefore, appear to be strongly in favour of formula II; but further investigations of the derivatives of alginic acid must be carried out before its constitution can be definitely established.

Experimental.

Action of Acetic Anhydride on Alginic Acid in the presence of various Catalysts.

Except where otherwise mentioned, the alginic acid used was prepared by the method already described (5).

I. *Pyridine.*—Some alginic acid jelly was soaked in pyridine and mixed thoroughly with it. The excess pyridine was poured off, and the residue was made into a thick paste with water. This was covered with a mixture of equal volumes of pyridine and acetic anhydride, and kept well stirred at a temperature below 40° C. for three days. The reaction mixture was then filtered, and the residue extracted with chloroform. Only a trace of substance soluble in this solvent was obtained. Acetone did not extract anything from the residue.

II. *Sodium Acetate.*—Dried and powdered alginic acid was treated with a large excess of fused sodium acetate and acetic anhydride, and boiled for ten minutes. The mixture was allowed to stand over-night, and heated again to boiling before pouring into a large volume of water. A white flocculent material separated, and the water became yellow in colour. The separated substance dissolved easily in acetone, with difficulty in alcohol, and was insoluble in chloroform. The yield was extremely small. 0·825 mg. required 0·334 c.c. of $N/100$ NaOH. Equivalent: 247. $C_6H_9O_7(COCH_3) = 236$.

III. *Zinc Chloride.* — 12 g. of crude commercial alginic acid, dried and powdered, was added to 200 c.c. of glacial acetic acid containing 12 g. of zinc chloride. The mixture was stirred mechanically, and 100 c.c. of acetic anhydride added over a period of 6 hours. The stirring was continued for 7 hours, and the mixture was heated for a further 2 hours to 80°. The alginic acid seemed unchanged. It was diluted with glacial acetic acid, and filtered quickly through linen. The clear filtrate was poured into a large volume of water, whereupon a white flocculent substance separated. This was filtered off and washed well with water. It was dissolved in chloroform, and was reprecipitated from this solvent by ether. Having been washed with ether it was dried in a vacuum desiccator, and finally at 100° in a vacuum of about 0·1 mm. It was a hard, cream-coloured, granular substance. Yield 0·8 g. 0·6376 g. required 8·1 c.c. of N.KOH for neutralisation and saponification.

Hence 315 g. required 4 litres of normal alkali. 320 g. of $C_6H_7O_7(COCH_3)_3$ (molecular weight, 320) would require 4 litres of normal alkali.

IV. *Iodine.*—A quantity of dry alginic acid was boiled intermittently with acetic anhydride containing a small amount of iodine. The clear liquid was decanted from the undissolved algin into a large volume of potassium iodide solution. A light yellow oil separated, which on stirring disappeared, leaving a light yellow paper-like material. This, when separated and washed with water, dissolved completely in acetone, from which it was obtained on evaporation as a light brown film. This was exhaustively extracted with boiling alcohol, from which a white flocculent substance (A) separated on cooling. Another product (B) was got by precipitation of the alcoholic filtrate with ether. Both were well washed with ether, and dried at 100° in a vacuum of 0·1 mm.

0·1082 g. of A required 2·80 c.cs. of *N*/2 KOH for neutralisation and saponification. Hence 77·2 gms. required 1 litre of normal alkali; 309 gms. required 4 litres. Molecular weight of triacetyl uronic acid: 320.

0·2182 gms. of B required 4·88 c.cs. of *N*/2 KOH for neutralisation and saponification. Hence 89·4 gms. required 1 litre of normal alkali; 268 gms. required 3 litres. Molecular weight of diacetyl uronic acid: 278.

V. *Hydriodic Acid.*—1 gm. of alginic acid in the form of jelly was boiled twice with alcohol and squeezed out. It was then covered with a mixture of glacial acetic acid and concentrated hydriodic acid in the proportions of 20 to 1 by volume and heated on the water bath for 15 minutes. The excess liquid was now poured off and 10 c.cs. of acetic anhydride were added. A vigorous reaction set in at once and much heat was developed. The mixture was cooled in ice and in a few minutes all but a trace of the alginic acid had gone into solution, and, on standing for a short time the contents of the flask set to a jelly. This was poured into ether and stirred. A white colloidal solid resulted and this was separated by the centrifuge and washed several times with ether. Yield: 0·6 gm.[1]

The above acetylated product was extracted several times with acetone, and the combined extracts after filtration were poured into ether. A white colloidal substance (A) separated out.

The residue from the acetone extraction was extracted with industrial spirit, and the extract after filtration was poured into ether. A white colloidal substance (B) separated out.

The residue insoluble in industrial spirit was made into a jelly by the addition of a little water, and on addition of industrial spirit the greater part of it went into solution, leaving a small residue (D). When the industrial spirit after filtration was poured into ether, a white colloidal substance (C) separated.

The substances A, B, and C were redissolved in their respective solvents

[1] In later experiments with careful cooling 60 per cent. of the theoretical yield was obtained.

and reprecipitated with ether. They were filtered off, and dried in a high vacuum.

The yield of A was 0·108 gm. It was an extremely light, cream-coloured powder. When partly dried it was soluble in acetone, in alcohol, in benzene-alcohol, and water. When dried in a high vacuum it was insoluble in acetone and in alcohol, and soluble with difficulty in water. A concentration of 1·25 per cent. can, however, be obtained in the latter. The aqueous solution is clear, and reduces Fehling's solution on boiling. Ferric chloride gives a light brown colloidal precipitate, and lead nitrate gives a slight precipitate. Silver nitrate, copper sulphate, barium nitrate, and calcium chloride cause no precipitation. The dried substance has no melting point. It begins to change at about 100°. The specific rotatory power of the free acid A was determined, with the following results:—

$$(1)\quad [\alpha]^{8}_{D}(H_2O) = \frac{-2{\cdot}05 \times 100}{1{\cdot}101 \times 2} = -93{\cdot}1°.$$

$$(2)\quad [\alpha]^{8}_{D}(H_2O) = \frac{-2{\cdot}32 \times 100}{1{\cdot}24 \times 2} = -93{\cdot}8°.$$

Carbon and Hydrogen.[2]—4·530 mgm. gave 6·830 mgm. CO_2, and 2·090 mgm. H_2O. Carbon, 41·12 per cent.; hydrogen, 5·12 per cent.. Calculated for diacetyl uronic acid: carbon, 43·16; hydrogen, 5·04 per cent.

Neutralisation and Saponification.—(1) 0·1775 gm. required 6·25 c.c. of *N*/10 NaOH for neutralisation and 12·5 c.c. extra for saponification. Equivalent from neutralisation, 284. Equivalent from total alkali, 284. Percentage of acetyl, 30·29.

(2) 0·2362 gm. required 8·26 c.cs. of *N*/10 NaOH for neutralisation and 17·67 extra for saponification. Equivalent from neutralisation, 285. Equivalent from total alkali, 275·4. Percentage of acetyl, 31·09.

Calculated for diacetyl uronic acid—equivalent, 278; percentage of acetyl, 30·93.

The yield of B was 0·19 gm. It is a white, granular powder, and has no melting point, beginning to change at 110°. When partly dried it dissolves in spirit to an opalescent solution, and is also soluble in benzene-alcohol and water, but insoluble in acetone and chloroform. When dried in a high vacuum it becomes insoluble in all organic solvents. It still dissolves with difficulty in water, forming an opalescent solution. This solution reduces Fehling's solution slightly on boiling. Ferric chloride gives a light brown gelatinous precipitate, copper sulphate a pale blue gelatinous precipitate, barium chloride or ferrous sulphate a turbidity, and silver nitrate no precipitate.

The specific rotatory power of the sodium salt of B was determined:

$$[\alpha]^{8}_{D}(H_2O) = \frac{-2{\cdot}32 \times 100}{1{\cdot}018 \times 2} = -113{\cdot}9°.$$

[2] Analyses marked with a [2] were by Dr. Schoeller of Berlin.

Carbon and Hydrogen.[2]—4·829 mgms. gave 7·255 mgms. CO_2, 2·250 mgms. H_2O, and 0·018 mgms. of ash. Found: carbon, 41·12 per cent.; hydrogen, 5·19 per cent.

Neutralisation and Saponification.—(1) 0·2486 gm. required 8·69 c.cs. of *N*/10 NaOH for neutralisation and 17·81 c.cs. extra for saponification. Equivalent from neutralisation, 285·7. Equivalent from total alkali, 281·4. Percentage of acetyl, 31·15.

(2) 0·1554 required 5·49 c.cs. of *N*/10 NaOH for neutralisation and 10·66 c.cs. extra for saponification. Equivalent from neutralisation, 279. Equivalent from total alkali, 284·8. Percentage of acetyl, 29·9. For calculated values for diacetyl uronic acid see above.

The yield of C was 0·252 gm. It is a light grey granular powder, and has no melting point. It is soluble in aqueous alcohol if first made into a jelly with water. The dried acetate is insoluble in all the solvents tried with A and B, including water. On boiling with Fehling's solution for a few minutes a slight reduction is noticeable. The solubilities of its salts are similar to those of B.

The specific rotatory power of the sodium salt of C was determined:

$$[\alpha]_D^{8°C.}(H_2O) = \frac{-1·017 \times 100}{0·3345 \times 2} = -136·8°.$$

Carbon and Hydrogen.[2]—5·112 mgms. gave 8·155 mgms. of CO_2, 2·430 mgms. of H_2O, and 0·020 mgms. of ash. Found: carbon, 43·68, hydrogen, 5·33 per cent.

Neutralisation and Saponification.—(1) 0·2508 gm. required 9·09 c.cs. of *N*/10 NaOH for neutralisation and 18·04 c.cs. extra for saponification. Equivalent from neutralisation, 274·7. Equivalent from total alkali, 277·2. Percentage of acetyl, 30·93.

(2) 0·3360 gm. required 12·03 c.cs. of *N*/10 NaOH for neutralisation. Equivalent, 279.

For calculated values for diacetyl uronic acid see above.

Constants of the De-acetylated Alginic Acids.—The solutions of the sodium salts of alginic acid resulting from the saponification of A, B, and C were used to determine the specific rotatory power of the de-acetylated acids. The results obtained were as follows:—

$$\text{A. } [\alpha]_D^8 = -96·5°. \quad \text{B. } [\alpha]_D^8 = -110·6°. \quad \text{C. } [\alpha]_D^8 = -122·1°.$$

The barium salts of the de-acetylated acids were then precipitated from the solutions by adding the calculated quantity of barium chloride. They were washed free of chlorine and dried in the usual manner used to determine the equivalents of the acids. The results were as follows:—

A. 0·1070 gm. gave 0·0396 gm. of barium carbonate. Equivalent, 198·8.
B. 0·1692 gm. gave 0·0734 gm. of barium sulphate. Equivalent, 201.
C. 0·1648 gm. gave 0·0747 gm. of barium sulphate. Equivalent, 191.

The insoluble residue D amounted to 0·05 gm. 0·0474 gm. was dissolved in 2·15 c.c. of $N/10$ NaOH. 0·029 c.c. of $N/10$ HCl was required to neutralise the excess alkali. Equivalent, 226. Equivalent of monoacetyl uronic acid, 236.

Summary.

Alginic acid was treated with acetic anhydride in the presence of various catalysts.

With sodium acetate and with pyridine very small yields of acetyl derivatives were obtained, and with zinc chloride a small yield of what appeared to be a triacetate of a polymerised uronic acid.

With iodine a mixture of di- and tri-acetates of polymerised uronic acid appeared to be obtained.

Pretreatment with hydriodic acid in glacial acetic acid causes acetylation with acetic anhydride to take place rapidly, resulting in a 60 per cent. yield of 3 compounds all having the formula $(C_6H_9O_7)(COCH_3)_2$ and appearing to be acetates of alginic acid in varying degrees of polymerisation.

These facts lend support to the view that alginic acid is a polymer, not of a uronic acid anhydride, but of the complete uronic acid.

We have to thank the Industrial Research Council for a grant in aid of the work described in this and the previous paper, and the Royal Dublin Society for the use of the polarimeter with which the measurements described in these papers were made.

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No. 33.

METHOD OF INFECTION OF OAT GRAIN WITH *USTILAGO AVENAE* AND THE INFLUENCE OF EXTERNAL FACTORS ON THE INCIDENCE OF THE DISEASE.

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(Communicated by Professor P. A. Murphy.)

(Plate 7.)

[Read March 24. Published separately July 13, 1936.]

Introduction.

From the publication of Jensen's paper (5) in 1888 until 1922 it was believed that infection of oats by loose smut was due to spores carried on the grain and between the glumes, especially those in the latter position, these spores germinating simultaneously with the grain when it was sown, and giving rise to infection. During this period it was known, however, that oats from a badly smutted crop when used for seed did not always show high percentages of disease; and also, when grain was contaminated just before sowing, very little infection occurred as a rule unless the hulls were removed beforehand.

Falck (3) obtained 50–80 per cent. of smutted plants by means of blossom inoculation with loose smut in 1908, and he observed not only the germination of some of the spores on the stigma of the blossoms, but also found mycelium subsequently penetrating the outer layers of the seed. Falck did not consider this fact of primary importance, because he found numerous ungerminated spores present on the surface of the grains as well as on the inside of the glumes, and tests showed these spores fully capable of germination.

In 1922, Zade (10) rediscovered the fact that during the flowering period of the oat the spores of loose smut germinate on the stigma and anthers as well as elsewhere in the floral parts, and he pointed out that these germinated spores form a resting mycelium, chiefly in the tissues of the outer glumes, and that this resting mycelium is the source of infection of the oat plant. Gage (2) working in America confirmed Zade's results; but he believed that most of the resting mycelium lay in the outer tissues of the caryopsis and not in the

glumes, and like Zade he attributed very little infection to ungerminated spores. Sampson (8) working in Wales also found that some of the smut spores germinate in the oat flower and form a resting mycelium, as described by Zade. Sampson records the following significant observation:—"Germination counts were not possible, but it was evident that a relatively small number of the spores introduced between the pales had germinated." When such flower-inoculated grain and contaminated shelled grain were sown under the same conditions she found that sometimes one method and sometimes the other gave the most smut, and she pointed out that the meagre results sometimes obtained in the latter case could be accounted for by the low viability of the spores employed for contamination.

It has been found in Ireland that when oat flowers are inoculated with loose smut during the flowering period, or immediately following it, a certain number of the spores germinate, and produce a resting mycelium, mainly in the glumes and to a less extent in the outer tissues of the caryopsis. The amount of such spore germination has always been low, and when this flower-inoculated grain was examined during the following winter viable ungerminated spores were always found. Examination of naturally infected oats also revealed resting mycelium in the glumes as well as spores capable of germination.

Following a discussion of the above facts with Professor Murphy early in the year 1933 it was believed that some useful additional information might be obtained from further investigation of loose smut of oats, and, accordingly, the following experiments were planned and carried out during the years 1933–'34–'35:—

Experiments with Loose Smut of Oats, 1933–'34.

In the year 1933 a plot of Potato oats was grown in isolation in order to carry out flower inoculation with loose smut (*Ustilago Avenae*). At the time of inoculation in July, 1933, an odd flower was fully open, but in the majority of cases fertilization had occurred, and the ovary was just beginning to swell. The anthers were exposed by cutting off the tips of the outer glumes with scissors, and inoculation was carried out by means of a camel-hair brush covered with spores. This removal of the glume tips is generally adopted by plant breeders when crossing oats; it is much quicker than opening the glumes and pales with forceps, and no injury occurs, as the grain develops normally afterwards. When the tips of the outer glumes are cut off soon after fertilization it is seen that the outer and inner glumes are not in close contact, and on gently tapping the brush the spores penetrate to all the floral parts, including the ovary, style, anthers, and inner pale. Any flowers on the panicle which were not inoculated individually were removed. During the time of inoculation the weather was dull and cloudy with occasional light rain. Two separate samples of loose smut were used, one lot of panicles being done with a pure line strain of smut, *Ustilago Avenae* L. 2. a. The second group of panicles was

inoculated with a mixed sample of loose smut collected in 1930 on Potato oats, and, therefore, capable of causing infection on this variety. Both samples were three years old, and though germination was moderately good in sugar solutions it was extremely poor in distilled water.

After the grain was threshed, examination showed that ungerminated smut spores could be obtained without difficulty by removing the glumes and inner pales and gently washing them with distilled water in a watch-glass, most of the spores being obtained from the inner pales. Some of these spores were still viable notwithstanding the age of the samples.

Previous to sowing in January, 1934, each lot of flower-inoculated grain was divided into four parts, which were treated as follows:—

1. No treatment.
2. Grain with hulls intact washed very vigorously 6 days before sowing underneath a tap for ¾ of an hour and then dried rapidly. Subsequent examination revealed ungerminated smut spores within the hulls.
3. Grain de-hulled by hand.
4. Grain de-hulled by hand and then washed and dried as in treatment 2.

A few days before sowing, clean Potato oats with hulls intact were contaminated with part of the smut samples left over from time of flower-inoculation.

In preparation for sowing ordinary field soil mixed with a little leaf-mould had been dried. The two samples of smutted grain were sown in flower-pots on different days, and in each case the washed grain was sown first, then the de-hulled grain, followed by flower-inoculated seed which received no treatment, and finally, grain contaminated just previous to sowing. After covering the seed, each group of pots in the experiment was divided into two—one-half being kept in their initial dry state and the other thoroughly saturated with water. The pots were then placed in an unheated room and covered until the first plants appeared. When all pots in each experiment had some plants visible they were removed to a cold greenhouse and watered. As soon as all plants were up the pots were removed outside, where they remained until the plants were finally transplanted in the open ground.

Further details of the experiment are shown in Table 1.

Experiments with Loose Smut of Oats, 1934–'35.

The percentages of smut obtained in 1934 were comparatively small, especially in the case of grain contaminated just before sowing, due to the age and low germination of the smut used. However, the results were consistent

TABLE 1.

Method and Time of Inoculation.	Subsequent treatment of seed.	Soil moisture content immediately after sowing.	*Ustilago Avenae*, L. 2. a.				*Ustilago Avenae* (mixed strains).			
			No. of grain sown.	Days before appearance of first and last plants.	No. of plants grown to maturity.	Smutted plants.	No. of grain sown.	Days before appearance of first and last plants.	No. of plants grown to maturity.	Smutted plants.
						Per cent.				Per cent.
Flowering period, 1933.	None.	50 % saturation	60	18—25	57	8	75	15—22	75	20
Do.	,,	Saturated.	60	16—26	58	3	75	14—18	75	0
Do.	Washed with hulls intact.	50 % saturation.	60	18—26	59	8	75	15—24	75	12
Do.	,, ,, ,,	Saturated.	60	17—24	60	5	75	14—24	74	1·5
Do.	De-hulled by hand.	50 % saturation.	60	17—23	60	3	75	14—23	73	13
Do.	,, ,,	Saturated.	60	16—23	55	2	75	13—18	75	0
Do.	De-hulled by hand and washed.	50 % saturation.	60	17—25	56	3	75	15—28	59	1·5
Do.	,, ,, ,,	Saturated.	60	15—23	57	0	75	14—16	75	0
Contaminated with dry spores, Jan., 1934.	Hulls intact.	50 % saturation.	60	18—25	60	8	75	16—28	74	7
Do.	,, ,,	Saturated.	60	16—21	60	0	75	13—21	75	0

Germination of *Ustilago Avenae*, L. 2. a., at time of flower inoculation, July, 1933, 16 per cent. in 24 hours, and 45 per cent. in 48 hours. Germination of smut at time of grain contamination, January, 1934, 10 per cent. in 24 hours, and 35 per cent. in 72 hours.

Germination of *Ustilago Avenae* (mixed strains), July, 1933, 20 per cent. in 24 hours, and 50 per cent. in 48 hours. Germination of this smut in January, 1934, 14 per cent. in 24 hours, and 25 per cent. in 72 hours.

At time of sowing soil moisture 50 per cent. of saturation capacity.

Temperature where pots were kept during 25 days following sowing:—Min. 6° C., Max. 10° C., corresponding approximately to spring temperatures, when oats are usually sown.

All grain sown 15th and 16th January, 1934. Depth of sowing, 1½ inches.

Plants transplanted to open 16th March, 1934. Panicles began to appear 155 days after sowing, smut 9 days later. Variety of oats, Potato.

in both cases, and it was decided to repeat the work on a larger scale in 1934. The smut used in 1934 was collected in each plot and inoculated into healthy plants in the same plot. The spores were stripped from a number of smutted panicles into a wide-necked bottle. Previous to inoculation the healthy panicles had the extreme tips of the glumes removed by a pair of scissors as in 1933. The panicle was bent over carefully and inserted into the bottle. By holding the stem of the panicle between the fingers, and at the same time using them for covering the mouth of the bottle, and giving the latter a slight shake, a cloud of spores was set in motion, which penetrated to all parts of the floral organs as before.[1] This method of inoculation is much quicker than doing the flowers individually with a camel-hair brush; it can, however, only be used where plenty of material is available.

At the time the grain was threshed some of the chaff was stirred up with water in a test tube, and the spores so obtained, which showed no signs of previous germination, germinated to the extent of 3–4 per cent. Washings of the cleaned grain also gave plenty of spores, but little germination. When, however, the inner pales were removed from a number of grains and placed in a weak sugar solution 8–9 per cent. germination of the adhering spores occurred inside 48 hours, both in October, 1934, and January, 1935. In January, 1935, the flower-inoculated grain from each plot was divided into four lots, which received similar treatments to those given in 1934. Washing was vigorous, and was carried out for one hour six days before sowing. The grain with the intact hulls and that de-hulled by hand were washed together in the same vessel, and after drying the two were separated, and each was placed in a clean envelope.

For sowing purposes a number of boxes had been prepared, each capable of holding 200 grains. Two lines were marked around the inside of each box, one, half-an-inch from the top of the box, and the other line one-and-a-half inches below the first, which insured that all grain was sown at the same depth. The soil used for the boxes was ordinary field soil of pH 7·6–7·8, and was artificially dried before using. The flower-inoculated grain was sown first, and in the same order as in 1934. Finally, the last seed sown in each experiment was Potato oats from a smut-free crop, which had been contaminated in part with hulls intact and in part with hulls removed ten days before sowing, the smut used being that left over from the time of flower-inoculation. The treatment of the boxes after sowing was similar to that given to the pots in 1934, with the exceptions that the initially dry boxes received a light watering on the fourteenth day after sowing, and the boxes were placed directly outside as soon as plants were visible.

Full details of the experiment are given in Table 2.

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[1] A number of panicles inoculated in a similar way, but without removal of the glume tips, gave equally good results when the grain was sown under dry conditions in 1935.

TABLE 2.

Method and Time of Inoculation.	Subsequent treatment of seed.	Soil moisture content immediately after sowing.	*Ustilago Avenae*, L. 2. a.				*Ustilago Avenae* (mixed strains).			
			No. of grain sown.	Days before appearance of first plants.	No. of plants grown to maturity.	Smutted plants.	No. of grain sown.	Days before appearance of first plants.	No. of plants grown to maturity.	Smutted plants.
						Per cent.				Per cent.
Flowering period, 1934.	None.	45 % saturation.	200	15	185	26	200	14	182	25
Do.	„	Saturated.	200	12	193	5	200	11	192	7
Do.	Washed with hulls intact.	45 % saturation.	200	16	192	14	200	16	187	11
Do.	„ „ „	Saturated.	200	14	189	4	200	13	187	4
Do.	Dehulled by hand.	45 % saturation.	200	16	184	13	200	15	193	14
Do.	„ „	Saturated.	200	14	185	3	200	13	192	6
Do.	Dehulled and then washed.	45 % saturation.	200	16	185	6	200	14	187	1
Do.	„ „ „	Saturated.	200	14	179	2	200	13	180	2
Contaminated with dry spores, Jan., 1935.	Hulls intact.	45 % saturation.	200	16	156	29	200	16	156	37
Do.	„ „	Saturated.	200	14	170	2	200	13	173	5
Do.	Hulls removed before contamination.	45 % saturation.	200	15	159	97	200	14	156	95
Do.	„ „ „	Saturated.	200	14	186	24	200	11	169	52

Germination of *Ustilago Avenae*, L. 2. a. at time of flower inoculation, July, 1934, 36 per cent. in 24 hours and 50 per cent. in 60 hours. Germination of smut at time of grain contamination, January, 1935, 20 per cent. in 24 hours and 40 per cent. in 48 hours.

Germination of *Ustilago Avenae* (mixed strains), July, 1934, 18 per cent. in 24 hours and 45 per cent. in 60 hours. Germination of this smut, January, 1935, 25 per cent. in 24 hours and 44 per cent. in 48 hours.

At time of sowing, soil moisture 45 per cent. of saturation capacity.

Temperature of room during first 18 days after sowing—Min. 6° C., Max. 11° C.

All grain sown 28 and 29 January, 1935. Depth of sowing, 1½ inches.

Plants transplanted to open 7–9 March, 1935. Panicles appeared 143 days after sowing and first smutted head 48 hours later.

Variety of oats, Potato.

Observations on the Diseased Plants in 1935.

Conditions for infection and development of the parasite in 1935 must have been exceptionally favourable. This is shown not only by the high percentage of smut occurring in each experiment, but also by the fact that sporulation occurred in the top leaves of a considerable number of plants as well as in the panicles. The sporulation of *Ustilago Avenae* on the foliage has been described by Bartholomew and Jones (1), and Sampson (8) records it for *Ustilago levis,* Faris (4) for *Ustilago Hordei,* and Viennot-Bourgin (9) for *Ustilago Tritici.* It had previously been seen in infected plants grown in a greenhouse, but this was the first occasion it was met with outside. Of the 369 plants infected with *Ustilago Avenae* L. 2. a. 32 per cent. showed black streaks on the leaves, as did 22 per cent. infected with the mixed strains of smut. The smut was confined generally to the top leaf, which was frequently twisted up into a spiral at the tip (see Plate), but in some cases as many as five of the uppermost leaves were involved. Plants with smut sori on their foliage occurred indiscriminately throughout both plots, irrespective of the method of inoculation or of the treatments. Notwithstanding this, plants with healthy and smutted panicles also occurred in every lot, the percentage of these being 24·6 in the pure line strain of smut, and 21·6 in the mixed strains. Infection was most complete in the case of plants kept under initially dry conditions as compared with initially wet conditions, the figures being 79 and 83 per cent. of plants wholly smutted for the former, and 67 and 69 per cent. for the latter, respectively, for the two smuts.

Smut Infected Grain Sown in the Open, 1935.

A small quantity of the flower-inoculated grain kept from each of the experiments described in Table 2 was sown in the open at a depth of 1½ inches on the 9th March, 1935, beside the transplanted plants, with the following result:—

(*a*) Flower-inoculated July, 1934, with *Ustilago Avenae* L. 2. a. 300 grains sown produced 3 per cent. smutted plants.

(*b*) Flower-inoculated July, 1934, with mixed strains *Ustilago Avenae.* 300 grains sown gave 2 per cent. smutted plants.

These results should be compared with the percentages shown in lines 1 and 2, Table 2.

In February, 1935, a sample of the variety Sandy oat was received from Mr. Denneny, of the Department of Agriculture in Co. Donegal. The crop from which this sample was obtained was badly smutted in 1934, and examination of the seed when received showed that it contained abundance of spores of loose smut. Washings from the grain were somewhat disappointing, as few spores were found in this way. Removal and examination of the inner

pales, however, showed plenty of spores present, varying in number from 4 to 22 per pale. From nine inner pales examined microscopically a total of 76 spores were obtained, which gave a germination of 34 per cent., and in some cases 50 per cent., of the spores germinated inside 48 hours. Only one of these nine pales gave spores which failed to give some germination. In this connection it may be pointed out that the previous August was showery throughout, rain falling on 19 days, presumably providing conditions suitable for germination. An attempt was made to reproduce smutty oats by sowing this seed outside. For this purpose a small plot of ground was deeply trenched and ridged up early in February, so that it would dry out, the plot being protected by a temporary covering to keep the rain off in the meantime. Unfortunately, this plot was too well dried, as at sowing time, on 22nd March, the soil moisture content of the top fifteen inches was only 33 per cent. of its saturation capacity. The covering was replaced on the plot after sowing, and remained on for ten days. The conditions were too dry, and of the 1,500 grains sown only 670 plants resulted, of which 1·3 per cent. were smutted. In an adjacent plot of untreated ground which was sown at the same time with 2,000 seeds of the same lot of oats, a practically complete stand was secured, which developed 0·9 per cent. of smut. These smut counts are too small to draw any conclusions from, but it is perhaps significant that all diseased plants in the dried-out plot were completely smutted, whereas three only in the other plot were so diseased, the other sixteen being partially smutted. The very low percentage of smut in the untreated plot is evidently due to the comparatively high moisture content of the soil; this was 52 per cent. at time of sowing, but heavy rain fell inside twelve hours. The result is typical of what has always occurred on the Glasnevin farm when oats from a naturally diseased crop are sown, for little or no disease develops.

TABLE 3.—SUMMARY OF RESULTS.

Results in Dry Soil.		Per cent. smutted plants.			
		1934.		1935.	
		L. 2. a.	Mixed.	L. 2. a.	Mixed.
Flower contamination.	No treatment.	8	20	26	25
	Washed—Hulls intact.	8	12	14	11
	De-hulled only.	3	13	13	14
	De-hulled and washed.	3	1·5	6	1
Seed contamination.	Hulls intact.	8	7	29	37
	Hulls removed.	–	–	97	95

Discussion of Results.

Zade and Arland (11) point out that under their conditions at sowing time ungerminated spores are not of any appreciable importance, because the viable spores germinate almost entirely at blossoming time, and they state: "Exact investigations have shown in noteworthy manner that almost all spores—indeed all, without exception—that are found between the glumes and caryopsis are non-viable." This is not so of certain strains of loose smut of oats occurring in Ireland, as examinations made during the winter and spring months both of artificially flower-inoculated oats and grain from naturally smutted fields have shown the presence of viable ungerminated spores in sufficient numbers to account for any resulting infection in the succeeding crop.

Spores adhering to the outside of the glumes are obviously functionless in the case of flower-inoculated oats, for the method of inoculation shows that internal spores or their products are the important factor. The great reduction in smut following the washing of de-hulled grain, especially in the mixed strain, can be most reasonably explained by actual removal of the spores (see summary of results, Table 3). If the main source of infection is resting mycelium in the tissues of the caryopsis or elsewhere and not adhering spores, as Gage (2) maintains, then it is difficult to see how mere washing with water could reduce the infection. De-hulling alone would be likely to reduce the number of spores, and the number of infected plants in the resulting crop bears this out.

The reduction in infection following washing hulled grain is more difficult to explain. Presumably it acted by removing some of the spores from within the glumes, and it is possible that this was facilitated by the absence of the glume tips. At all events, it is hard to understand what effect washing could have on resting mycelium. As would be expected, washing flower-inoculated grain does not eliminate all ungerminated spores.

An examination of Table 3 also indicates that washing was less effective in the case of L. 2. a. in reducing smut in both years. This was true of washing both hulled and de-hulled seed, and it may indicate that certain strains of smut tend to survive in the form of resting mycelium rather than spores. This would explain the discrepancies in previous work.

Grain with the hulls intact and contaminated previous to sowing with loose smut of a high germination, gave as high percentages of infection as flower-inoculated oats when sown under the same conditions (see Table 2). Any comparison of flower-inoculated grain, however, should only be made with grain de-hulled previous to contamination, as the introduction of spores into the oat flowers is really equivalent to de-hulling, and then contaminating with spores, as in both cases the spores are introduced direct to the surface of the caryopsis. As regards the spore load carried on grain contaminated previous to sowing, this was undoubtedly heavy, both in the case of grain de-hulled and

grain with hulls intact. At the same time, an examination of the flower-inoculated grain showed that it also carried an excessive spore load, owing to the method of inoculating, much greater by far than would ever occur under natural conditions.

Most investigators (1, 6, and 7) agree that a relatively dry soil and moderately high temperature favour infection by loose smut of oats. Sampson (8) found that in weekly sowings made under natural conditions, rainfall rather than temperature was the critical factor. The present experiments support this view. Irrespective of whether the source of infection was *resting mycelium* or spores, a low soil moisture content immediately after sowing was the principal factor operating in the infection of the host plant. In only one instance did a wet soil give a higher percentage of smut than the corresponding dry soil; and the figures here are so small, 2 per cent. and 1 per cent. respectively, that no significance can be attached to them.

Summary.

Loose smut of oats was used for flower inoculation at and immediately following the flowering period, and the same samples of smut were again used for contaminating grain, both with hulls intact and with hulls removed, previous to sowing. Grain inoculated in these different manners was sown under the same conditions, and the amount of infection compared in two successive years.

In the first year flower-inoculated oats gave the highest percentage of disease in a single experiment only. In the second season, under the experimental conditions described, and using fresh spores of high germination, shelled grain contaminated before sowing gave the highest percentage of infection, followed by that with hulls intact and then flower-inoculated oats.

De-hulling flower-inoculated oats reduced infection by from 35 to over 50 per cent. when such grain was sown.

Washing grain with hulls intact, derived from flower-inoculated oats, also reduced infection by from 32 to 56 per cent. when such grain was sown.

De-hulling and washing flower-inoculated oats reduced infection by from 62 to 79 per cent. in the case of a pure line strain of loose smut, and by from 90 to 96 per cent. in the case of mixed strains of *Ustilago Avenae*.

From the foregoing it is concluded that in the case of flower-inoculated oats, and the particular strains of loose smut worked with, most of the infection was due to ungerminated spores within the glumes and not to resting mycelium.

Examination in the winter months of artificially flower-inoculated oats and of grain from smutted crops occurring naturally, revealed both resting mycelium and viable ungerminated spores within the glumes, sufficient of the latter being present to account for any infection appearing in the following crop.

Acknowledgments.

This work was carried out in the Division of Plant Pathology, Albert Agricultural College, Glasnevin, under the direction of Professor P. A. Murphy, to whom I am also indebted for help and criticism in the preparation of the manuscript. Thanks are due to Mr. M. Caffrey, Lecturer in Plant Breeding, and his assistant, Mr. P. T. Carroll, for facilities to plant the experimental plots in isolation each year. Acknowledgment is also made to Mr. Denneny, Agricultural Overseer, Co. Donegal, who kindly supplied naturally infected oats in the spring of 1935.

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Explanation of Plate 7.

Oat plant showing spiral twist of tip of uppermost leaf and sporulation of loose smut on the leaf-blade as well as in the panicle.

No. 34.

THE INHIBITION OF CHEMICAL REACTIONS. PART 6. THE INFLUENCE OF ETHER AND NITROBENZENE ON THE ABSORPTION OF ETHYLENE BY SULPHURIC ACID.

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[Read May 26. Published separately July 14, 1936.]

In Part 5 of the present series (Sci. Proc. Roy. Dublin Soc. (1932), **20**, 267) it was pointed out that, according to Davis and collaborators (J. Amer. Chem. Soc. (1930), **52**, 721, 3769), when ethylene gas is absorbed by sulphuric acid the reaction appears to take place entirely in a thin film at the surface of the liquid. The chief evidence for this belief was that, when the sulphuric acid was stirred from below without disturbance of the liquid surface, the rate of absorption of ethylene was unaffected. Further, no sign of rapid initial absorption of ethylene was observed, such as might have been expected if ethylene enters into ordinary solution in the sulphuric acid prior to reaction.

It had been suggested previously by Bailey that the inhibition by foreign substances of reactions between a liquid and a gas probably takes place in many cases at or near the surface of the liquid; and in Part 5 Hickson and Bailey sought for inhibitors of the ethylene-sulphuric acid reaction in the hope of finding evidence for or against this hypothesis. A number of retarding agents was discovered, and the action of water and pyridine was examined in detail. Surface tension measurements, however, showed that these substances raise the surface tension of sulphuric acid, and that they are, therefore, present in the surface layer in concentrations lower than in the bulk of the liquid.

The present research was undertaken in the hope of finding substances which would be surface-active and at the same time act as inhibitors of the reaction. If such substances could be found, an attempt might be made to trace a connection between their surface concentration and their retarding effect.

A search of the literature revealed the fact that ether lowers the surface tension of sulphuric acid (Pound, J. Chem. Soc. (1911), **99**, 710), and it was found by one of the writers that nitrobenzene has a similar effect. Surface tension curves have now been made for the whole range of mixtures of sulphuric acid with (*a*) ether and (*b*) nitrobenzene (Bailey, J. Chem. Soc., 1936, 684), and the results for mixtures containing small proportions of ether or nitrobenzene are used in the present work.

The estimation of the actual surface concentration of a solute is a matter of much difficulty and uncertainty. Direct measurements have been made in certain cases, but where one is dealing with a corrosive, hygroscopic liquid, such as 100 per cent. sulphuric acid, such measurements are almost out of the question. The calculation of surface concentration is usually made from surface tension by means of the Gibbs equation.

$$\Gamma = -\frac{Cn}{RT} \cdot \frac{d\sigma}{dC},$$

where Γ = excess of solute in the surface layer (molecules per sq. cm.),
C = bulk concentration of solute (gm. molecules per c.c.),
σ = surface tension (dynes per cm.),
T = absolute temperature,
R = the gas constant ($83{\cdot}15 \times 10^6$ ergs per gm. molecule),
and $n = 6{\cdot}06 \times 10^{23}$.

When Γ is added to the normal surface concentration of solute, as calculated from the bulk concentration, the total surface concentration is obtained.

Unhappily, in cases where a direct determination of surface concentration has been found possible, the surface concentration determined experimentally does not always agree with that calculated from the Gibbs equation, and the values in the latter case are frequently too low. Calculation from surface tension gives, however, the nearest approximation to the truth that seems obtainable in the present work, and in the tables which follow the total surface concentrations have been thus calculated.

The measurements of the rate of absorption of ethylene were made by methods similar to those used in Part 5, and the details of surface tension measurements have been recorded elsewhere by Bailey (*loc. cit.*). The values used for σ and for $\frac{d\sigma}{dC}$ are obtained from the latter paper, and are not reproduced here. The temperature of most experiments was 17°, and where the temperature was slightly different corrections were employed to adjust results to 17°.

The velocity (V_0) of absorption of ethylene by pure sulphuric acid has been taken as 100, and velocities (V_I) in the cases where the acid contained an inhibitor are recorded as percentages of V_0. ($V_0 - V_I$) is the "percentage retardation."

Both ether and nitrobenzene caused considerable retardation, one molecular proportion of ether in four hundred of acid cutting down the reaction velocity by 14 per cent., and a similar concentration of nitrobenzene cutting it down by 11 per cent.

When the determinations were complete, it was clear that the inhibiting effect of these substances was not a simple function of their concentrations in the bulk of the liquid. The total surface concentration was then evaluated by means of the Gibbs equation, and in the case of ether the result was striking.

In Fig. 1 the bulk concentration of the retarders has been plotted against (*a*) their calculated surface concentration, and (*b*) the percentage retardation caused by them. By choosing appropriate scales the two ether curves can be made to coincide almost exactly, while the points for the surface concentration of nitrobenzene lie far from the retardation curve. It seems as if the mechanism of nitrobenzene inhibition must be different from that of ether inhibition.

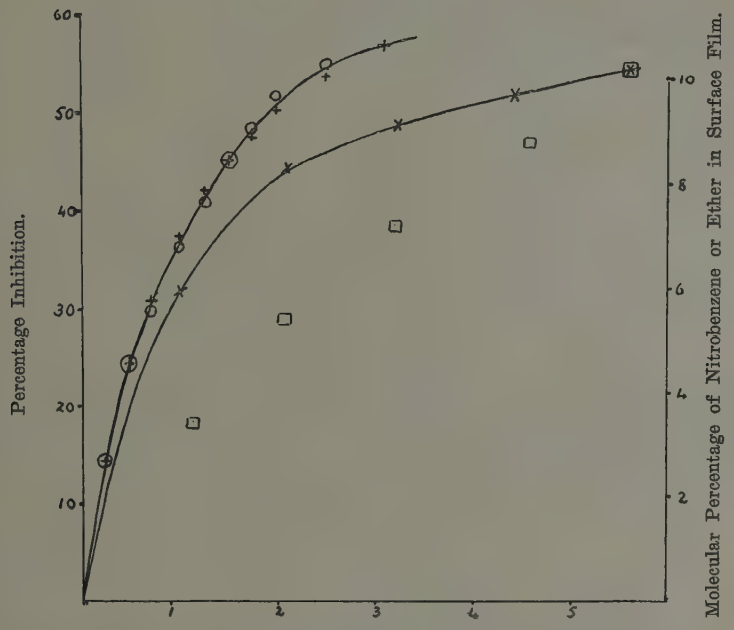

Fig. 1.

\+ + + Percentage Inhibition by Ether.
× × × Percentage Inhibition by Nitrobenzene.
° ° ° Molecular Percentage of Ether in Surface Film.
□ □ □ Molecular Percentage of Nitrobenzene in Surface Film.

In the case of ether, every molecule in the surface layer seems to have equal inhibiting power, the ratio (percentage retardation/molecular percentage ether in surface layer) being about 5·3. If the surface concentration of ether is correctly given by the calculation, it appears as if a molecule of ether in the liquid surface can not only refuse to absorb an ethylene molecule impinging on it, but can prevent four or five neighbouring molecules of sulphuric acid from doing so. It is possible that an ethylene molecule, to be absorbed, must make

contact with two adjoining sulphuric acid molecules (such cases are known where solid catalytic surfaces are concerned), and that replacement of one of these by ether results in failure to absorb.

No such relatively simple mechanism will explain the nitrobenzene retardation, as is clear from Fig. 1. Let us consider whether a mechanism based on formation of an intermediate complex, which may either re-arrange itself into final products, or be dislocated by collision with a retarding molecule, will give an equation in accord with experimental data.

The following series of reactions is suggested:—

(1) $C_2H_4 + H_2SO_4 = (H_2SO_4 . C_2H_4)$ (k_1).

(2) $(H_2SO_4 . C_2H_4) = C_2H_5 . HSO_4$ (k_2).

(3) $(H_2SO_4 . C_2H_4) + C_6H_5 . NO_2 = C_2H_4 + H_2SO_4 + C_6H_5NO_2$ (k_3).

On Bodenstein's theory of stationary concentration, in a reaction with such a mechanism, proceeding with a moderate velocity, the rate of change of concentration of the intermediate complex $(H_2SO_4 . C_2H_4)$ must, after the first few seconds, be small (approximately zero). Otherwise the reaction velocity would increase or diminish rapidly.

Let x = molecular percentage of retarder, and $100 - x$ be molecular percentage of acid at the site of reaction, i.e., in the surface layer or in the body of the liquid, according to the theory adopted. The reaction velocities have been calculated for a constant pressure of ethylene, which can be included in the constant k_1. Then

$$\frac{d\,[\text{complex}]}{dt} = 0 = k_1\,(100 - x) - k_2\,[\text{complex}] - k_3\,(x)\,[\text{complex}].$$

$$\therefore \quad [\text{complex}] = \frac{100 - x}{k_2/k_1 + k_3/k_1\,(x)}.$$

But the reaction velocity is really the rate at which the final product is formed from the intermediate complex in reaction (2), and is, therefore, proportional to [complex].

$$\text{The velocity, } \therefore, = k_v . \left(\frac{100 - x}{k_2/k_1 + k_3/k_1\,(x)}\right) = \frac{100 - x}{k_2/k_1k_v + k_3/k_1k_v\,(x)}.$$

If we decide arbitrarily (as we have done) to consider V_O (the velocity when $x = 0$) as 100, it is clear that k_2/k_1k_v must $= 1$. The velocity, therefore, becomes $\dfrac{100 - x}{1 + Bx}$, where $B = k_3/k_1k_v$.

We may now apply this equation by taking the experimental velocity, using the known value of x in the bulk of the liquid (or the calculated value in the surface layer), and calculating B. Let B_L be the value of the constant when reaction in the bulk of the liquid is assumed, and B_S when the reaction is

supposed to take place in the surface layer, and the calculated surface concentration of nitrobenzene is employed.

Table 1.

Nitrobenzene as retarder.

Molecular per cent. nitrobenzene in bulk of liquid.	Molecular per cent. in surface layer (calculated).	V_I	B_L	B_S
0·16	0·65	93	0·46	0.11
0·38	1·37	85·4	0·44	0·11
0·55	1·74	80·6	0·42	0·13
1·1	3·2	68·8	0·40	0·13
2·2	5·4	57	0·33	0·12
3·35	7·3	50·2	0·28	0·12
4·5	8·7	48·7	0·21	0·10
5·6	10·1	45·7	0·19	0·10

It is clear that the values of B_L show no approach to constancy, while those of B_S are fairly constant. The equation

$$V_I = \frac{100 - x}{1 + \overline{Bx}},$$

therefore, gives satisfactory results if the values of x are the surface concentrations of nitrobenzene, but not if they are the bulk concentrations. Nitrobenzene, therefore, probably retards by dislocating an intermediate complex at or near its site of formation in the surface layer before it can rearrange itself into the final product.

Similar calculations for ether as retarder are given in the following table:—

Table 2.

Ether as retarder.

Molecular per cent. ether in bulk of liquid.	Molecular per cent. in surface layer (calculated).	V_I	B_L	B_S
0·25	2·66	86	0·64	0·050
0·5	4·6	75·7	0·63	0·057
0·75	5·56	68·8	0·59	0·067
1·0	6·62	62·4	0·58	0·075
1·25	7·59	58·1	0·56	0·078
1·5	8·37	55	0·53	0·080
1·75	9·07	52·5	0·50	0·081
2·0	9·64	49·9	0·48	0·084
2·5	10·20	48·5	0·44	0·091

Neither B_L nor B_S is constant, and we are confirmed in our belief that the retardation by ether is different in kind from that by nitrobenzene, and is probably passive rather than active in character.

While the calculations just given provide reason for believing that the inhibitors exercise their retarding action at the gas-liquid interface, more direct evidence is desirable, and the following experiments on lines suggested by those of Adeney and Becker (Sci. Proc. Roy. Dublin Soc., 1918, **15**, 385; 1919, **15**, 609) seem to provide such evidence.

If a bubble of ethylene is passed up a long tube full of sulphuric acid the liquid surface exposed to the ethylene is constantly renewed as the acid streams down over the surface of the rising bubble. If a surface-active inhibitor is present in the sulphuric acid, it will not have time to reach in such a rapidly-changing surface the equilibrium attained in a static surface.

From Fig. 1 we can read off the percentage inhibition, $100\left(1 - \frac{V_I}{V_O}\right)$, produced in a static experiment by a given concentration of ether or nitrobenzene. If these substances retard in the body of the liquid the ratio of the velocities V_I / V_O should be much the same if bubbles of ethylene are allowed to ascend (*a*) a column of sulphuric acid containing inhibitor, and (*b*) a similar column containing none, although the absolute velocities in the bubble experiments will be very much higher than in the static experiments. If, however, ether and nitrobenzene retard by virtue of their accumulation at the surface of the liquid, the percentage inhibition in the bubble experiments should be much smaller than in the static experiments.

To minimise experimental error it is desirable that the bubble should traverse the length of the tube a considerable number of times. With 100 per cent. sulphuric acid, absorption of ethylene from the moving bubble was found to be so rapid that a high degree of accuracy could not be attained, and most of the experiments were carried out with acid containing from 5 to 0·5 per cent. water. The tube used was 80 cm. long and 6 mm. wide, with a tap at either end, graduated at the end at which the ethylene was admitted. The length of the bubble was measured as rapidly as possible. The bubble was passed up the tube a suitable number of times, and the tap was opened, atmospheric pressure being thus restored. The final length of the bubble was measured.

Let n = number of lengths of tube traversed,
A = per cent. ethylene absorbed,
I_B = per cent. inhibition in bubble experiments,
I_S = per cent. inhibition in static experiments (read from Fig. 1).

Table 3.

Conditions.	Inhibitor.	A	I_B	I_S
95 per cent. H_2SO_4	None	34	—	—
$n = 20$.	Ether (3 per cent.) . .	35	nil	57
98 per cent. H_2SO_4	None	35	—	—
$n = 14$.	Ether (0·86 per cent.) . .	35	nil	33
	Nitrobenzene (1·2 per cent.) .	34	3	35
99·5 per cent. H_2SO_4.	None	51	—	—
$n = 6$	Ether (1·3 per cent.) . .	45	12	42
	Nitrobenzene (0·93 per cent.) .	51	nil	30
100 per cent. H_2SO_4.	None	79	—	—
$n = 4$	Nitrobenzene (2·64 per cent.) .	70	11	52

While the experimental error in such experiments is certainly high, it seems clear that, when the surface is constantly renewed, the retarding agent has very much less effect than when the surface is stationary. This fact offers strong support to the theory that the inhibitor acts at the gas-liquid interface, and not in the body of the liquid.

The senior author is very much indebted to Professor F. E. Hackett, University College, Dublin, for drawing his attention to the experiments by Adeney and Becker, and for suggesting that experiments on similar lines might throw light on the present problem.

Summary.

1. The rate of absorption of ethylene by sulphuric acid is diminished sharply by the presence of small quantities of ether or nitrobenzene.

2. No connection could be traced between the bulk concentration of either retarder and its inhibiting power.

3. The inhibiting power of ether was directly proportional to its concentration at the surface of the liquid, as calculated by means of the Gibbs equation.

4. The velocity of the reaction in presence of nitrobenzene was given by the equation

$$V_I = \frac{100 - x}{1 + Bx},$$

where x is the concentration of nitrobenzene at the surface of the liquid.

5. Experimental evidence is given that these retarders are relatively inefficient if the liquid surface is moving too rapidly for the retarder to accumulate there.

6. Both retarders, therefore, appear to exercise their influence in the film at the surface of the liquid. Their probable modes of action have been discussed.

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